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Cover Photograph: Mississippi diamondback terrapin (*Malaclemys terrapin*), a nesting female from Cedar Point Marsh, Alabama.

Photo is courtesy of: Dr. Andrew T. Coleman, Institute for Marine Mammal Studies, Gulfport, Mississippi.

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EFFECT OF BY-CATCH REDUCTION DEVICES (BRDS) ON THE CAPTURE OF DIAMONDBACK TERRAPINS (*MALACLEMYS TERRAPIN*) IN CRAB POTS IN AN ALABAMA SALT MARSH

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ABSTRACT

By-catch is a serious conservation threat to populations of numerous marine species, including the diamondback terrapin. Terrapins are top level predators in their estuarine environments and can play an important role in maintaining salt marsh integrity. Unfortunately, numerous populations have declined due to drowning in submerged commercial and recreational crab pots. By-catch reduction devices (BRDs) were developed to prevent terrapin by-catch without affecting crab capture, and they have been shown in previous studies to be effective. The current study examined the efficacy of BRDs in reducing terrapin by-catch in a depleted population of terrapins along the Gulf Coast of Alabama. The BRDs significantly decreased terrapin by-catch (an approximate 90% reduction). There was no significant reduction in the overall capture of crabs in BRD-equipped pots; however, capture of marketable-sized crabs was significantly lower in pots modified with BRDs. This latter finding was in contrast to several large scale studies that had much larger sample sizes and found no significant reduction in the capture of marketable-sized crabs with the use of BRDs. The results indicate that BRDs represent an effective and practical management tool in reducing terrapin by-catch in commercial and recreational crab pots in Alabama.

INTRODUCTION

The impacts of by-catch from marine fisheries have been well documented and have become a conservation concern for a variety of species such as sea turtles (Hall et al., 2000; Lewison et al., 2003; Pinedo and Polancheck, 2004; Gilman et al., 2006; Moore et al., 2009), sharks (McKinnell and Seki, 1998; Beerkircher et al., 2002; Campana et al., 2009), seabirds (Melvin et al., 1999; Lewison and Crowder, 2003; Gilman et al., 2005; Moore et al., 2009) and marine mammals (Cox et al., 1998; Read et al., 2006; Moore et al., 2009). These species are adapted to K-selected traits such as long life spans and delayed sexual maturity; therefore, the survival status of these species is particularly susceptible to by-catch-induced mortality of adults and/or sub-adults.

The diamondback terrapin (*Malaclemys terrapin*) is an exclusively estuarine turtle whose range extends from Cape Cod, MA, along the Atlantic and Gulf Coasts to Corpus Christi, TX, (Ernst and Lovich, 2009) and displays similar life history traits (Gibbons, 1987). Unfortunately,

many populations have experienced declines due to a variety of natural and anthropogenic threats. Major threats include crab pot-induced mortality (Roosenburg et al., 1997; Wood, 1997; Roosenburg, 2004) as well as habitat loss (Roosenburg, 1991), nest predation (Lazell and Auger, 1981; Feinberg and Burke, 2003; Draud et al., 2004), and road mortality (Wood and Herlands, 1997; Szerlag and McRobert, 2006). In the case of crab pot-induced mortality, juvenile and adult terrapins represent by-catch that are attracted to bait, captured blue crabs (*Callinectes sapidus*), or other by-catch. Once in the submerged crab pots, terrapins will drown unless the pot is checked soon after the terrapins are captured, especially in oxygen-depleted waters during the summer months (Roosenburg et al., 1997; Wood 1997; Roosenburg, 2004).

The proliferation of the blue crab fishery along the Atlantic and Gulf coasts of the U.S. from the middle of the 20th century to present day (Kennedy et al., 2007) has decimated terrapin populations because the range of terrapins greatly overlaps with the habitat that is heavily fished for the blue crab resource. Roosenburg et al. (1997) described how devastating crab pot-induced mortality can be to a population in Maryland. Based on both trapping and mark recapture data, the authors estimated that crabbing could potentially remove 58-78% of the terrapin population annually. Even if the estimates were overstated by a factor of four, crab pot-induced mortality still could result in annual mortality of 20% of the population (Roosenburg et al., 1997).

A long-term mark recapture study by Dorcas et al. (2007) indicated that crab pot-induced mortality was a major factor causing the decline of a terrapin population inhabiting the salt marshes of Kiawah Island, SC. The decline was accompanied by a shift in age structure to older individuals and a shift in sex ratio to more females, which would be the predicted impact of crab pots (Dorcas et al., 2007). Terrapins exhibit sexual dimorphism, with adult females growing to a much larger size than adult males (Lovich and Gibbons, 1990), to account for divergent reproductive priorities (Gibbons et al., 2001). As a consequence, adult males do not attain a size that would exclude them from entering crab pots (Roosenburg et al., 1997). Crab pots were indicated as the primary factor causing the decline and demographic changes in the Kiawah Island population, including both the commercial as well as the recreational crab fishery (Hoyle and Gibbons, 2000; Dorcas et al., 2007). Roosenburg et al. (1997) also suggested that crab pot-induced mortality was the cause of the female-biased sex ratio observed in their Maryland population.

Not only do regularly fished pots pose a danger to terrapins, but so do abandoned pots that are termed "derelict" or "ghost pots." Derelict pots have been found to contain numerous terrapin carcasses: 49 in one pot in Maryland (Roosenburg, 1991) and 133 in two pots in Georgia (Grosse et al., 2009). Based on mark-recapture estimates for the tidal creek from which the two pots were recovered in Georgia, these dead individuals represented approximately double the number of remaining live terrapins in that population (Grosse et al., 2009). In the Gulf of Mexico, yearly crab pot loss can be quite high (20-100%) (Guillory et al., 2001) and has been conservatively estimated to be approximately 250,000 derelict pots per year (Perry et al., 2003). Thus, the threat that abandoned pots pose in the Gulf of Mexico region is substantial.

In addition to the direct mortality of by-catch, authors have suggested that the ecological effects of the discarded by-catch need to be examined. Both noncommercial and commercial species rely on a functioning ecosystem, and the removal or severe reduction of certain species can greatly alter this requirement (Hall et al., 2000; Kennelly and Broadhurst, 2002). Recent research has indicated that the diamondback terrapin can represent a keystone predator in its salt marsh habitat (Silliman and Zieman, 2001; Silliman et al., 2005; Gustafson et al., 2006). A major prey item is the periwinkle snail (*Littoraria irrorata*), and these snails have been shown to

decimate salt marsh vegetation through their fungal farming activities (Silliman and Zieman, 2001; Silliman et al., 2005; Gustafson et al., 2006).

Modifications to crab pots have been developed in an effort to decrease terrapin mortality. Roosenburg et al. (1997) changed the overall shape and size of crab pots by removing the top of the pot and adding PVC and netting so that the top of the pot remains above the water, which would allow captured terrapins to reach the surface to breathe. However, these larger pots may be only practical for recreational crabbers to use (Roosenburg et al., 1997). Wood (1997) developed a by-catch reduction device (BRD), which is a metal or plastic rectangle and fits into the funnel openings of the pot. It reduces the size of the openings to theoretically prevent terrapin entry without affecting crab capture. This inexpensive modification potentially represents a more appropriate modification to commercial crab pots to decrease terrapin by-catch.

The implementation of an effective strategy for facilitating the recovery of the diamondback terrapin requires the evaluation of specific conservation measures in the region of interest (Butler and Heinrich, 2007). The purpose of the current study was to assess the effectiveness of BRDs in reducing terrapin captures in crab pots in the salt marshes of Alabama. Diamondback terrapins were once an abundant and economically important species in Alabama, but the state now lists the terrapin as a species of "highest conservation concern" (Mirarchi et al., 2004). In addition to the capture of terrapins, the study also evaluated the effect of BRDs on the capture of marketable-sized blue crabs. Both datasets provide insight on whether or not BRDs represent an effective and practical method for decreasing crab pot-induced mortality of diamondback terrapins in the salt marshes of Alabama.

MATERIALS AND METHODS

We conducted a side-by-side comparison of crab pots fitted and not fitted with BRDs from May to August in 2007-2009. We placed eight pairs of pots at various locations in Cedar Point Marsh (N 30° 19' 33.70", W 88° 08' 36.36"). The Alabama population of diamondback terrapins exists in isolated remnant aggregations, and the largest one, identified to-date, inhabits Cedar Point Marsh, which has been extensively studied starting in 2004 (Coleman, 2011). Cedar Point Marsh is dominated by black needle rush (*Juncus roemerianus*), salt marsh cord grass (*Spartina alterniflora*), and coastal salt grass (*Distichlis spicata*). Invertebrates and vertebrates inhabiting the marsh include fiddler crabs (*Uca panacea*), periwinkle snails, striped mullet (*Mugil cephalus*), Atlantic croaker (*Micropognias undulates*), and Southern flounder (*Paralichthys lethostigma*). Because we are working with a depleted population of terrapins, we utilized the modified crab pot design of Roosenburg et al. (1997). The BRDs were metal rectangles measuring 5.08cm x 15.24 cm (2" x 6"), and we placed the BRDs midway into the funnel openings. We checked the pots and baited them with menhaden at least twice a week during the sampling periods.

We estimated carapace width (point to point) of captured crabs to the nearest inch and released them, unless they were kept for bait. For the terrapins that were captured, we collected a suite of morphological measurements (Table 1). We determined the sex of each terrapin based on relative length between the edge of plastron to the vent (Lovich and Gibbons, 1990). To identify recaptures, we attached a shell tag with a unique identification number to the terrapin's carapace along with a PIT (passive integrated transponder) tag inserted into the musculature of the left hind limb.

Table 1. Morphological measurements taken from each captured terrapin

Straight-line carapace length (SLCL)	Straight-line carapace width (SLCW)
Plastron length (PL)	Shell depth (SD)
Weight	

We ran two sample paired t-tests at an α level of 0.05 to observe any significant differences in catch per unit effort (C.P.U.E.) (capture pot^{-1} day^{-1}) for terrapin capture, marketable-sized crab (> 5" or 127mm) capture, and total crab capture between the two pot types. We also ran two sample paired t-tests assuming unequal variance on each terrapin morphological parameter to test for differences between terrapins captured by the two pot types as well as to test for differences between male and female captured terrapins. We utilized the Data Analysis package in Microsoft© Excel for each analysis.

RESULTS

Terrapin By-catch

We sampled Cedar Point Marsh for a total of 153 trap days over the three years, and the overall terrapin capture for both pot types was extremely low. We captured 22 terrapins in non-BRD pots and 2 in BRD pots. The mean weekly C.P.U.E. for terrapin capture between the two pot types was significantly different (t=-2.178, p=0.033) (Figure 1). Two female terrapins comprised the BRD pot catch, and four males and 18 females were captured in non-BRD pots. Six of the non-BRD pot captures were recaptures from the various surveys (modified crab pots, pitfall traps, and otter trawl) employed by Coleman (2011). Utilizing the same criteria for designating mature females (a PL of 14.8 cm and a weight of 705 g) as Lovich and Gibbons (1990), five of the 18 captured females were adult females. Additionally, seven of the captures in non-BRD pots occurred in one capture event in June 2009.

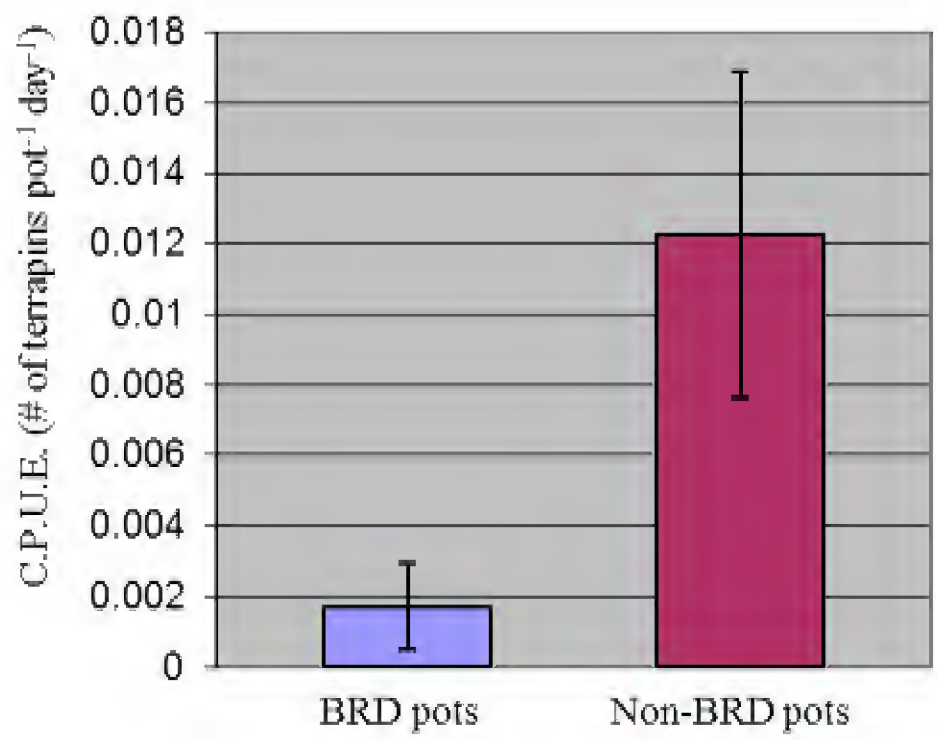

Figure 1. Catch per unit effort (C.P.U.E.) of terrapins captured pot^{-1} day^{-1}. We observed a significant difference between pot type (t=-2.178, p=0.033).

Only SLCW was significantly different between terrapins captured in BRD pots and those captured in non-BRD pots (Table 2). However, all five morphological parameters were significantly different between male and female captured terrapins (Table 3).

Table 2. Mean, standard deviation, and standard error for morphological parameters measured for each captured terrapin by pot type. Only straight line carapace width was significantly different between the two groups (t=-3.290, p=0.0167).

	SLCL (cm)	SLCW (cm)	PL (cm)	SD (cm)	Weight (g)
w/BRDs (n=2)					
Mean	13.36	9.65*	12.19	5.6	412.25
St. Dev.	0.49	0.36	0.58	0.14	46.02
St. Error	0.35	0.26	0.41	0.1	32.54
w/oBRDs (n=22)					
Mean	14.41	10.98*	12.96	5.9	521.73
St. Dev.	1.98	1.48	2.01	0.8	219.42
St. Error	0.42	0.31	0.43	0.17	46.78

Table 3. Mean, standard deviation, and standard error for morphological parameters measured for each terrapin by sex. The means for each parameter were significantly different between the two groups (SLCL: t=-4.197, p=0.0007; SLCW: t=-5.635, p<0.0001; PL: t=-6.228, p<0.0001; SD: t=-5.641, p<0.0001; Weight: t=-5.833, p<0.0001)

	SLCL (cm)	SLCW (cm)	PL (cm)	SD (cm)	Weight (g)
Male (n=4)					
Mean	12.5*	9.15*	10.63*	5.05*	283.26*
St. Dev.	0.62	0.42	0.35	0.13	26.22
St. Error	0.31	0.21	0.17	0.07	13.11
Female (n=20)					
Mean	14.69*	11.21*	13.35*	6.05*	558.48*
St. Dev.	1.88	1.35	1.79	0.73	202.7
St. Error	0.42	0.3	0.4	0.16	45.32

Crab Catch

Overall, non-BRD crab pots caught more marketable-sized crabs and total crabs. Non-BRD pots caught a total of 525 crabs, with 216 of those being of marketable size (5" or 127 mm) versus 383 total crabs and 149 marketable-sized crabs in BRD pots. This equated to a 27% and 31% reduction in total crab capture and marketable-sized crab capture, respectively, in BRD pots. However, we did not observe a significant difference in the mean weekly C.P.U.E. for total crab capture (t=-1.147, p=0.256) (Figure 2) between the two pot types whereas a significant

difference in the mean weekly C.P.U.E. for marketable-sized crab capture (t=-2.788, p=0.007) (Figure 3) between the two pot types was observed.

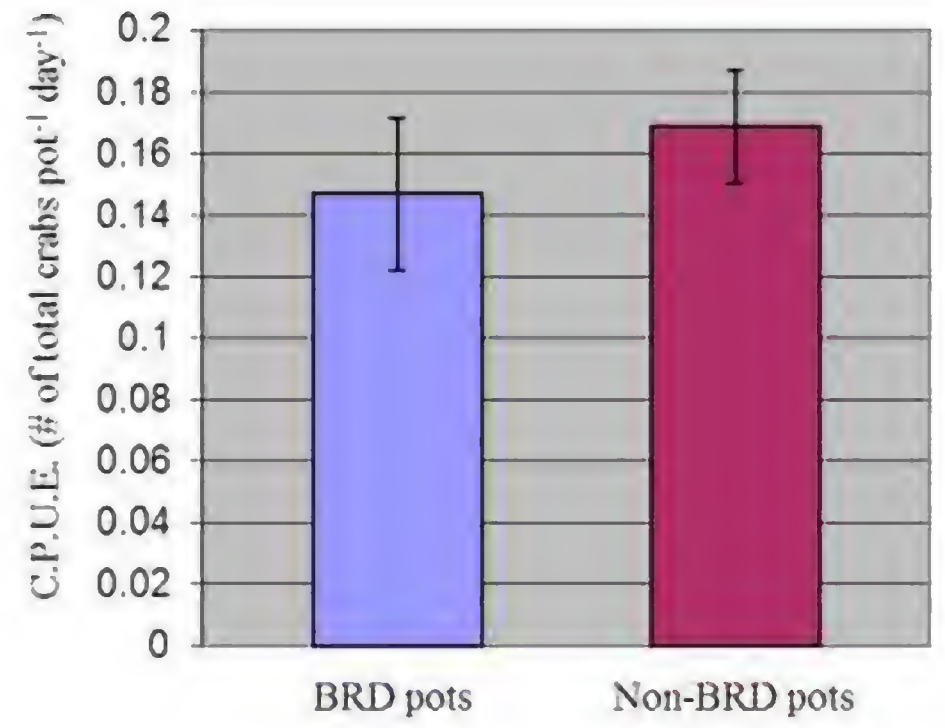

Figure 2. Mean weekly catch per unit effort (C.P.U.E.) for total crabs caught with BRD pots and non-BRD pots. No significant difference was observed for total crab C.P.U.E. between pot types (t=-1.147, p=0.256).

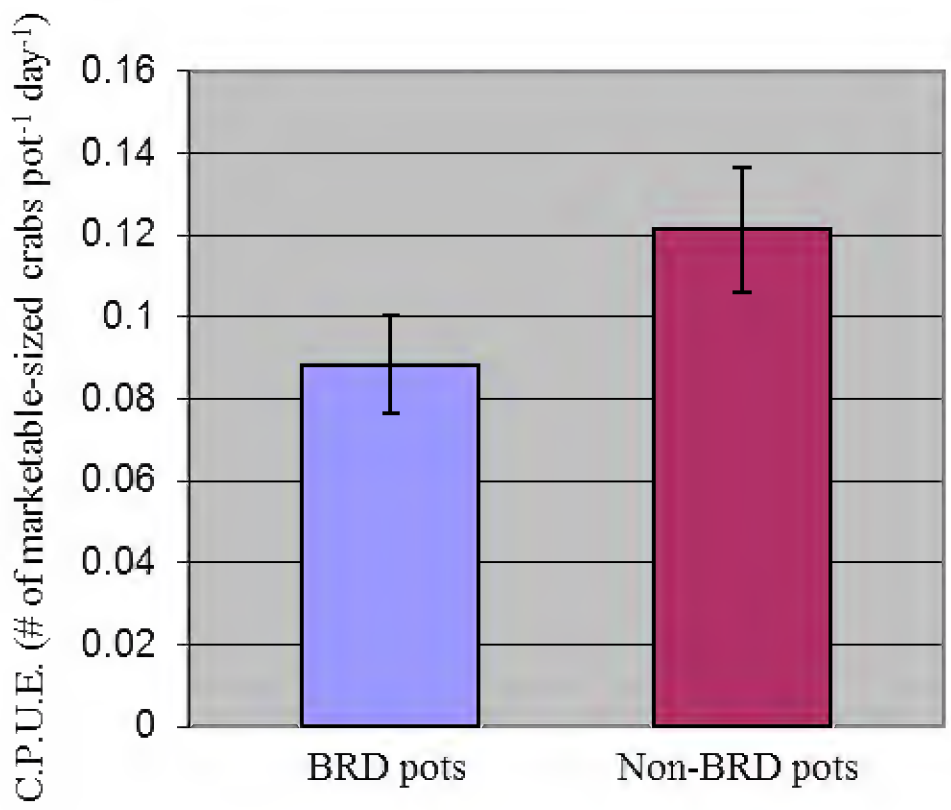

Figure 3. Mean weekly catch per unit effort (C.P.U.E.) of marketable-sized crabs caught with BRD pots and non-BRD pots. Non-BRD pots displayed a significantly higher C.P.U.E. (t=-2.788, p=0.007).

DISCUSSION

Kennelly and Broadhurst (2002) provided a framework to effectively address by-catch

issues. These steps include quantifying and identifying the by-catch, developing or improving gear modifications to reduce by-catch amounts and completing reliable field studies to test these modifications, and finally earning the approval of affected fisheries and other concerned interest groups. The issue of crab pot-induced mortality of diamondback terrapins is easily viewed within this framework, and the current study adds to the wealth of information indicating that BRDs can effectively reduce terrapin mortality. The current results extend these findings to the north central Gulf of Mexico and show that BRDs significantly reduce the capture of terrapins in crab pots in an Alabama salt marsh. Of the 24 terrapins caught in crab pots in Cedar Point Marsh, only two were captured in pots fitted with BRDs, suggesting an approximate 90% decrease in the capture of terrapins. The two terrapins that entered the modified pots must have distorted the BRDs because their shell height (5.5 cm and 5.7 cm) was more than the height of the BRDs (5.08 cm). Thus the use of a more rigid BRD or a smaller version of the BRD could potentially have prevented the capture of these two terrapins. A smaller version of BRD has been tested in previous studies (described below). Regardless, BRDs represent an effective means of significantly reducing terrapin by-catch in crab pots in Alabama.

BRDs have been shown to significantly inhibit terrapin pot entry in every study that has examined their efficacy. Wood (1997) and Roosenburg and Green (2000) both compared a variety of BRD sizes in crab pots in New Jersey and Maryland, respectively, and both found that using BRDs with a smaller height than 5 cm resulted in lower captures of terrapins than when the 5x10 cm BRD was employed. But, crab pots fitted with any size BRD caught significantly fewer terrapins than non-BRD pots (Wood, 1997; Roosenburg and Green, 2000). Butler and Heinrich (2007), Rook et al. (2010), and Morris et al. (2011) utilized 4.5x12 cm BRDs in their studies and observed the high efficiency of BRDs at excluding terrapins from crab pots. Morris et al. (2011) noted that 92% of all captured females and 70% of all captured males caught in non-BRD pots in their study would have been prevented from entering pots with the 4.5x12 cm BRDs.

While BRDs represent an effective means of decreasing the capture of terrapins, their benefit must be weighed relative to the potential negative impacts on crab capture rates. In the current study, there was no significant difference in the mean weekly C.P.U.E. of total crab capture, although we observed a 31% reduction in the number of marketable-sized crabs in the BRD pots. Interestingly, this finding contradicted the findings of a variety of larger-scale studies. The sample size of captured crabs in the current study (908) was low in comparison to other regional studies, whose sample sizes range from over 2,000 crabs (Roosenburg and Green, 2000; Butler and Heinrich, 2007) to over 20,000 crabs (Wood, 1997). Roosenburg and Green (2000) and Butler and Heinrich (2007) observed no significant differences in crab capture between pot types while Wood (1997) observed a higher crab capture in BRD pots. Additional studies reported similar results when using BRDs in crab pots (Guillory and Prejean, 1998; Cole and Helser, 2001; Rook et al., 2010; Morris et al., 2011). An ongoing long-term study by the Gulf Coast Research Laboratory (GCRL, Ocean Springs, MS) and the Mississippi Department of Marine Resources has utilized several commercial crab fishermen for evaluating the effect of BRDs on crab capture. With over two years of data collected and a total sample size of approximately 140,000 crabs, their results suggest that crab capture is not affected in pots fitted with BRDs (D. Graham, GCRL, pers. comm.).

In addition to the number of crabs, use of BRDs could influence the size of crabs. Rook et al. (2010) detected larger crab biomass and size in modified pots, and Roosenburg and Green (2000) noted the largest crabs were in the pots with 4.5x12cm BRDs. It has been speculated that the presence of the BRD may provide increased rigidity to the funnel opening, thus preventing

manipulation by the crabs and eventual egress (Guillory and Prejean, 1998; Roosenburg and Green, 2000). Further, the presence of live terrapins in a crab pot could decrease crab capture. Morris et al. (2011) observed that non-BRD pots that contained live terrapins had lower crab capture than other non-BRD pots that did not have captured terrapins.

The lower crab capture rate in BRD-fitted pots in the current study could be due to a variety of factors. The side-by-side arrangement of BRD versus non-BRD crab pots could have influenced the results. Roosenburg (2004) noted that a side-by-side comparison could affect crab behavior by permitting them to enter the more easily accessible non-BRD pot as opposed to a BRD pot. The study location could also have affected the results. It was chosen because Cedar Point Marsh represents one of the largest terrapin aggregations identified to-date in Alabama (Coleman, 2011); however, as exemplified by the low number of crabs captured in the current study, this may not be an optimal crabbing area. Crab fishermen rarely utilize this channel, although the surrounding bay is heavily trapped, and a nesting female that was tracked via radio transmitter traveled from Cedar Point Marsh through this surrounding bay to a neighboring marsh (Roberge et al., unpublished data). Therefore, pots in this bay could enhance the overall threat of crab pot-induced mortality to terrapins in the adjacent marshes.

If BRDs are adopted as a management strategy, the size of the BRD can affect the capture rate of terrapins as well as crabs. Roosenburg and Green (2000) concluded that in Maryland the 4.5x12 cm BRD performed the best at preventing terrapin while still allowing sufficient crab capture. This size BRD was also used in the studies completed by Butler and Heinrich (2007), Rook et al. (2010), and Morris et al. (2011). However, Wood (1997) found that the 5x10 cm BRD performed best in New Jersey, and this BRD was also utilized in the Guillory and Prejean (1998) study. For recreational crabbing, tall crab pots developed by Roosenburg et al. (1997) with an even smaller BRD (even though its crab C.P.U.E. is lower) might be most appropriate. Roosenburg and Green (2000) stated that comparison studies in specific regions would need to be performed to see which BRD would be most successful there. Based on the shell height data collected for all male terrapins captured in the larger population study (Coleman, 2011), approximately 60% were small enough to enter pots fitted with the size of BRDs (5x15 cm) utilized in this study, so a BRD with a smaller height would be more successful in preventing adult male terrapin by-catch. However, all sub-adult female terrapins captured in the present study would have been excluded with a 5x15 cm BRD, if properly installed.

A female-biased sex ratio was observed in captured terrapins. The finding of a female bias is consistent with results of our ongoing mark recapture study in Cedar Point Marsh (Coleman, 2011). This could be due to a variety of factors, including the effect of crab pots on terrapin population demographics. Crab pot-induced mortality has been shown to significantly alter population structure, resulting in a female-biased sex ratio (Roosenburg et al., 1997; Dorcas et al., 2007). Yet, Roosenburg et al. (1997) suggested the loss of juvenile females could be more detrimental to the survival status of the population because of the loss of their reproductive potential. As an example, a sub-adult female that was captured in a non-BRD pot in 2009 was recaptured on the nesting beach in 2010 (Coleman, pers. obs.). Moreover, our modified crab pots captured adult females, one of which was recaptured on the nesting beach in 2010 and 2011 (Coleman, pers. obs.). Therefore, crab pots in Alabama could have substantial negative impacts on the reproductive viability on terrapins by removing both juvenile and adult females. The low mean weekly terrapin C.P.U.E. in non-BRD pots in the current study (0.012 terrapins pot^{-1} day^{-1}) compared to other areas (0.17 terrapins pot^{-1} day^{-1} in Maryland (Roosenburg et al., 1997) and 0.20 terrapins pot^{-1} day^{-1} in Virginia (Rook et al., 2010)) suggested a small population size,

which is likely the result of the historic impact of the crab fishery (Wood, 1997) on the terrapin population in Alabama. Despite the low C.P.U.E., individual pots can have intense trapping events (Roosenburg, 1991; Grosse et al., 2009). This was relatively observed in the present study; of the 24 terrapins captured in non-BRD pots, seven of these were trapped in one pot on the same day in 2009. Hart and Crowder (2011) observed a terrapin C.P.U.E. in North Carolina comparable to the one we observed in Alabama but argued that crab pot-induced mortality could still represent a major threat in areas with low terrapin C.P.U.E.s if these values are extrapolated to the total number of crab pots fished in that area.

Another effective management strategy would be a consistent derelict crab pot removal program. In recent years, several Gulf Coast states have initiated such efforts with great success. The Derelict Trap Task Force under the Gulf States Marine Fisheries Commission authored a set of guidelines for the development of a derelict pot removal program (Perry et al., 2003). Havens et al. (2008) utilized side-scan sonar technology to aid in locating derelict crab pots in Virginia. Also, closing areas with known terrapin aggregations (e.g., Cedar Point Marsh) to crab pots would be an effective measure. If total closure of Cedar Point Marsh to crabbing is not a feasible option, time period restrictions in spring and early summer, which corresponds to the highest activity levels for terrapins (Gibbons et al., 2001), could prove to be another effectual management action (Hart and Crowder, 2011).

The issue of terrapin by-catch has met the first four steps of the framework described by Kennelly and Broadhurst (2002). Terrapin by-catch has been clearly defined and quantified as a major problem associated with the crab fishery. BRDs were developed and evaluated by reliable field studies to be successful at preventing terrapin by-catch. The last step in the framework is to involve the affected fishing industry (crab fishery) in the implementation process to gain their approval, a notion that has been proposed by several studies (Melvin et al., 1999; Hall et al., 2000; Gilman et al., 2005; Hall and Mainprize, 2005). As mentioned earlier, the researchers performing the on-going study in Mississippi examining the efficacy of BRDs are partnering with commercial crab fishermen to collect data from their pots (D. Graham, GCRL, pers. comm..). This type of synergistic relationship between science and industry will benefit the ultimate goal of facilitating the recovery of the diamondback terrapin population in Alabama.

CONCLUSIONS AND RECOMMENDATIONS

Crab pot-induced mortality is a major threat to the survival status of diamondback terrapins throughout their range, including Alabama (Seigel and Gibbons, 1995; Butler et al., 2006). The current study, along with a variety of past studies, has shown that the use of by-catch reduction devices (BRDs) significantly decreases terrapin capture in crab pots (an approximate 90% reduction in terrapin capture was recorded in the current study). Although Cedar Point Marsh represents one of the largest terrapin aggregations identified to-date in Alabama (Coleman, 2011), the low C.P.U.E. reflects the relatively small populations size. This exemplifies the need for an effective management plan for facilitating the recovery of diamondback terrapins in Alabama. The results of the current study indicate that the implementation of BRDs represents an effective method of alleviating crab pot-induced mortality in Alabama.

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A MOLECULAR SURVEY OF CILIATES FOUND IN SHADES CREEK, JEFFERSON COUNTY, ALABAMA

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ABSTRACT

Several strategies were used to determine the identity and number of ciliate species present in upper Shades Creek, an impaired stream, and one of its small tributaries in Homewood, Jefferson Co., AL. Live ciliates were collected from either tuna traps or raw water samples and maintained as cultures on rye grass medium inoculated with *Klebsiella pneumoniae.* DNA was extracted from some of these cultured ciliates. Also, following either centrifugation or filtration to concentrate microorganisms, DNA was extracted from environmental samples. Either an SSU rDNA segment or the cytochrome C oxidase I gene was amplified by PCR using ciliate-specific primers. All methodologies yielded ciliate DNA products. PCR-amplified DNA was cloned into *TOP-10* competent cells using the *TOPO-TA pCR2.1* vector (Invitrogen), inserts were sequenced, and a BLAST search was performed to identify the closest relative among published ciliate sequences. Seventeen different species of ciliates were found, including four members of the genus *Paramecium*, one of which is likely a new species. The centrifuged raw creek water samples yielded the greatest variety of ciliates. *Geneious Pro* software (Biomatters) was used for DNA data analysis and construction of phylogenetic trees. Live *P. multimicronucleatum* and *P. tetraurelia* are freely available from D. A. Johnson (djohnso2@samford.edu) upon request. The technique for the isolation of PCR-amplifiable DNA from a single freshwater ciliate is also described.

INTRODUCTION

Protists are important consumers in the freshwater food web (Heath *et al.,* 2003; Marxsen, 2006), and the ciliates are key in this web as primary consumers of bacteria. However, published freshwater ciliate community data is limited. In a survey using universal nuclear small subunit ribosomal DNA (SSU rDNA) primers and DNA sequencing, Slapeta *et al.* (2005) suggested that the diversity of freshwater eukaryotes, especially ciliates, may be much greater than previously described microscopically. In pools just south of Paris, France, they found that ciliates were the most abundant and diverse of the eukaryotes identified. Similar eukaryotic surveys (including ciliates) have been conducted on a lake in Bloomington, IN, USA (Dawson and Pace, 2002), a small river in Geneva, Switzerland (Berney *et al.*, 2004), and a river in Spain (Amaral Zettler *et al.*, 2002). Dopheide *et al.* (2008) describes the use of ciliate-specific SSU rDNA primers in the characterization of ciliates from four streams in Aukland, New Zealand. However, since ciliate

survey data in the USA are still lacking and molecular methodology provides the best picture of community diversity (Slapeta *et al.*, 2005), we describe in this report the beginning of the first detailed ciliate survey of a body of water in Alabama.

Eukaryotic surveys (including ciliates) have been conducted in various impaired environments including three sites cited above: anoxic sediments from a lake in Bloomington, IN, USA (Dawson and Pace, 2002), sediments from a small river in Geneva, Switzerland (Berney *et al.*, 2004), and an acidic river in Spain (Amaral Zettler *et al.*, 2002). Even within these degraded ecosystems, levels of diversity for protists remain high. Our study site, Shades Creek, is designated as an impaired body of water due to fecal coliform bacteria, sedimentation, and turbidity (USEPA, 2010). Many of the causes of impairment are related to urban land cover, and include storm sewers, construction, and impervious runoff (USEPA, 2010). Shades Creek's ecosystem instability due to the effects of urbanization makes it a stream of importance in understanding the effects of these factors on ciliate communities.

We present here the results of using various strategies that attempt to discover ciliates found in a freshwater source using two sampling techniques (tuna traps and raw-water sampling), concentration of organisms by centrifugation or filtration, then molecular biological identification. By DNA sequencing of the nuclear SSU rDNA and mitochondrial cytochrome C oxidase I (COX) gene, we identified the presence of 17 species of ciliates in upper Shades Creek and one of its small tributaries in Jefferson County, AL (Figure 1). Use of ciliate-specific primers rather than generic eukaryotic primers resulted in selection at the amplification step of ciliate DNA thus narrowing the search for these freshwater ciliates. In this report we also describe methodology for amplifying DNA from a single ciliate.

MATERIALS AND METHODS

Sample Collection

Samples were collected near Samford University from upper Shades Creek (a tributary of the Cahaba River) and one of its small tributaries that flows in a southeasterly direction through the Samford campus just east of Propst Hall. Sampling sites are indicated in Figure 1. This stretch of Shades Creek is rapidly flowing but with few rapids and has an average depth of approximately one meter. Water samples were taken from near the surface of very still pools or side waters. Tuna traps were placed at the bottom of similar shallow waters (less than approximately 20 cm deep).

Four sampling strategies, described in more detail below, were used: 1) setting tuna traps; 2) centrifugation of water samples; 3) filtration of water samples; 4) using unconcentrated raw creek water samples.

Tuna Traps. Tuna traps were constructed by placing about 4 cc of tuna in the bottom of a 50 mL disposable conical centrifuge tube and covering the opening with nylon hose material secured with a large rubber band. Traps were placed in shallow, still creek water and secured by burying the base in the soil or by covering it with rocks. Traps were collected after 2 to 4 days and examined immediately for the presence of ciliates and again after 1 to 3 days. Individual ciliates were isolated on sterile plastic petri dishes using a 10 µL mechanical pipettor under 30x magnification using a dissecting microscope. Cultures were established and DNA was isolated from these cultures as described below under "Ciliate Cultures." In Table 1, organisms collected by this method are indicated as "trap culture" in the column "Sampling."

Also, for DNA isolation from uncultured tuna trap water, organisms were concentrated by repeated centrifugations at 900 x g. The loose pellet was used for DNA isolation as described below. In Table 1, organisms identified by this method are indicated as "trap water" in the column "Sampling."

Centrifugation. For the second sampling technique, approximately 500 mL of water was collected from still creek water in a glass container that had been disinfected with 70% ethanol. The water sample was then heated to 70ºC in a microwave to kill and immobilize organisms. Samples were then divided into twenty-four 15 mL conical centrifuge tubes and centrifuged at 6,000 x g for 4 min. The loose pellets from the 24 tubes were distributed into two 15 mL tubes and centrifuged at 6,000 x g for 4 min. One of these two loose pellets was pipetted into a 1.5 mL microfuge tube and centrifuged at 14,000 x g for 4 minutes, the supernatant discarded, then the other loose pellet pipetted into the same 1.5 mL tube and centrifuged at 14,000 x g for 4 minutes. The pellet was used for DNA isolation as described below. In Table 1, organisms identified by this method are indicated by "centri. creek" under the column "Sampling."

In some cases, tuna trap water was concentrated by centrifugation as described above.

Filtration. For the filtration method, approximately 250 mL of raw stream water was filtered through a sterile 0.22 µm filter cartridge (Fisher) using a 50 mL disposable Leur-Lok syringe (Kendall), and the filtrate was discarded. The filter cartridge was opened by scoring around the perimeter where the upper and lower halves meet, then by tapping with a hammer. DNA was isolated from this filter as described below. In Table 1, organisms identified by this method are indicated as "filtration" under the column "Sampling."

Unconcentrated. In some cases, DNA was extracted directly from raw creek water. In Table 1, organisms identified by this method are indicated as "creek water" under the column "Sampling."

Ciliate Cultures

Cultures of ciliates were initiated by placing a single organism in rye tea. Rye tea was 3.5 g dry rye seed per L of water, which was boiled, cooled, then pH adjusted to 7.8-7.9 with $Na_2HPO_4 \cdot 7H_20$. The tea was then autoclaved, cooled, adjusted to pH 6.8-7.2 with HCl, and then stored at 4ºC. An aliquot of rye tea was inoculated with *Klebsiella pneumoniae* and cultured at room temperature overnight before use. Ciliate cultures were grown at room temperature or at 17ºC in sterile 10 mL or 50 mL plastic centrifuge tubes with an initial volume of 3 to 5 mL rye tea at room temperature with the screw cap attached loosely. Cultures were fed weekly by adding about 2 mL room-temperature bacteria-inoculated rye tea. When tubes were near full, 3 to 5 mL were transferred to a new tube and the process repeated.

DNA isolation

DNA was isolated using one of the following two methods.

The Chelex Method. For the DNA isolation from a single ciliate or from 3 to 5 ciliates, a modification of the chelex method of Regensbogenova *et al.* (2004) was used. A single ciliate was isolated with a 10 µL mechanical pipettor and washed in nuclease-free sterile water. The ciliate was then transferred to 50 µL of thoroughly mixed *InstaGene Matrix* (BioRad, 6% chelex solution), 5 µL of a 49:1 dilution of *LongLife Proteinase K* (G-Biosciences) was added, tubes were gently mixed then incubated at 55ºC for 30 min followed by heating at 98ºC for 5 min.

Tubes were placed on ice to cool then centrifuged at 3000 x g for 5 min. The supernatant was used immediately for PCR amplification.

In Table 1, organisms identified using this DNA isolation method are indicated as "chelex" in the column "DNA Isolation."

Wizard Genomic DNA Isolation Kit. The *Wizard Genomic DNA Isolation Kit* (Promega) was used as the other DNA isolation method. For isolation of DNA from tuna-trap water samples, the final supernatant described above "Centrifugation" was discarded, 600 µL of *Nuclei Lysis Solution* (Promega) was added, and the *Wizard Genomic DNA Isolation* protocol was followed. In Table 1, organisms identified by this method are indicated by "Wizard" under the column "DNA Isolation." When the "Wizard" method was used for isolation of DNA from 0.22 µm filter cartridges, the filter was broken into several small pieces and placed in a 1.5 mL tube with 600 µL *Nuclei Lysis Solution,* and the *Wizard Genomic DNA Isolation* protocol was followed.

PCR Amplification

DNA was amplified using the degenerate SSU rDNA (18S rDNA) primers 384F (5'-YTBGATGGTAGTGTATTGGA-3') and 1147R (5'-GACGGTATCTRATCGTCTTT-3')(Dopheide *et al.*, 2008) or the COX primers CoxL11058 (5'-TGATTAGACTAGAGATGGC-3') and CoxH10176 (5'-GAAGTTTGTCAGTGTCTATCC-3')(Barth *et al.*, 2006). These SSU primers were chosen because of their ciliate-specificity. We found that Paramecium DNA was not effectively amplified by these SSU rDNA primers, so the COX primers were also also used and proved more efficient at amplifying *Paramecium* DNA. PCR amplification was performed on either a *MiniCycler* (MJ Research) or a *MiniOpticon System* (BioRad) thermocycler using 33 cycles of 94ºC denaturation, 55ºC annealing, and 72ºC replication (50ºC annealing temperature was used for SSU rDNA amplification of *P. multimicronucleatum*). After confirmation of amplification by agarose gel electrophoresis, PCR products were cleaned up using *Qiaquick* spin columns (Qiagen).

Cloning and Sequencing

Cloning of PCR products was performed using the *TOPO-TA pCR2.1* cloning kit (Invitrogen). PCR products were ligated into *pCR 2.1* vectors and cloned into *TOP-10* competent cells following the recommended Invitrogen protocol using ampicillin selection and X-gal blue/white screening. At least 10 white colonies were picked per ligation reaction. These cultures were grown in LB amp broth overnight at 35ºC with shaking. In order to confirm the presence of the insert, 0.5 µL of each culture was used directly in a PCR amplification with the M13F(-21) and M13R vector primers. Agarose gel electrophoresis was used to confirm that the insert was of expected length. These cultures were streaked onto LB amp plates, which were sent to *Genewiz*, Plainfield, NJ for sequencing using the M13F(-21) and M13R vector primers. Alternatively, plasmids were isolated from transformed cells using the *Quantum Prep® Plasmid Miniprep Kit* (BioRad), and plasmids were sent to *DF/HCC DNA Resource Core* (Boston, MA) for sequencing.

DNA Sequence Analysis

Geneious Pro software (Drummond *et al.* 2010) and the NCBI BLAST site (http://www.ncbi.nlm.nih.gov/BLAST/) were used for DNA sequence analysis and construction of phylogenetic trees. Alignments were performed using the Geneious Alignment tool with a 65% similarity cost matrix (5.0/4.0) and free end gaps. Neighbor Joining trees were constructed using the Tamura-Nei distance model and 1000 bootstrap replicates with a 50% support threshold.

RESULTS

We developed a protocol for the isolation of DNA from a single freshwater ciliate for PCR-amplification. The DNA isolation protocol, based on the method of Regensbogenova (2004), is summarized in Materials and Methods under "The Chelex Method."

Ciliate DNA was obtained by all collection methods and all DNA isolation procedures. The 17 ciliate species found, their collection sites, the collection method, and the DNA isolation method are indicated in Table 1. SSU rDNA sequences have been submitted to GenBank under the accession numbers shown in Table 1 and are also shown next to the sample name of each ciliate description below. All isolates were named according to the sites where organisms were collected (sites A, B, C, D, E, and F: see Figure 1). The descriptions below are listed by these assigned sample names. Table 1 lists them in order of the presumed species of each isolate. Following DNA isolation, either the COX gene or SSU rDNA was PCR-amplified, cloned, and sequenced.

1) A1 (submitted to GenBank as JN232893). DNA of this ciliate was isolated using the Wizard method from centrifuged creek water samples. The SSU rDNA sequence showed 99.7% pairwise identity (616 out of 618 bases) with a published DNA sequence from *Chilodonella uncinata* (accession number AF300283) and 99.6% pairwise identity with another published *C. uncinata* sequence (AF300282).

2) A2, A3, A4, A5 (A2 submitted to GenBank as JN243999). DNA of this ciliate was isolated using the Wizard method from a centrifuged creek water sample. Three clones (A2, A3, and A4) yielded the same SSU rDNA sequence, which showed 98.6% pairwise identity (629/638 bases) with four different species of *Tetrahymena* (*T. tropicalis*, EF070260; *T. pyriformis*, X56171, EF070254, EF070255, and M98021; *T. silvana*, EF070257; and *T. setosa*, AF364041) as well as one unidentified *Tetrahymena* species (EF070263). All changes were substitutions. One other clone, A5, differed from the other four by a single transition.

3) C1, C2, C3, C4, C5, D1, D2, D3, D4, D5, E10, E12 (C1 and E12 submitted to GenBank as JN244000 and JN244004, respectively). DNA of all C site samples was isolated using the chelex method from a centrifuged pellet of tuna trap water. Four SSU rDNA clones from this sample (C1, C3, C4, C5) yielded an identical 637 base sequence. This sequence had 99.7% pairwise identity (637/639 bases) with a published DNA sequence from *Colpidium*

Table 1. Ciliates identified from Shades Creek, Homewood, Alabama

Sample	Best BLAST Match	Sampling Method	DNA Isolation	Primers	Accession #
A1	*Chilodonella uncinata*	centr. creek	Wizard	SSU rDNA	JN232893
C1	*Colpidium campylum*	centr. creek	Wizard	SSU rDNA	JN244000
E12		filtration	Wizard	SSU rDNA	JN244004

F1	*Euplotes eurystomus*	centr. creek	Wizard	SSU rDNA	JN244008
E11	*Hypotrichida sp.*	centr. tuna	Wizard	SSU rDNA	JN244003
	Pleurotricha lanceolata				
	Oxytricha sp.				
F2	*Lembadion bullinum*	centr. creek	Wizard	SSU rDNA	JN244009
F3	*Levicoleps biwae*	centr. creek	Wizard	SSU rDNA	JN244010
F6	*Loxophyllum spirellum*	centr. creek	Wizard	SSU rDNA	JN244012
E1	*Paramecium caudatum*	centr. creek	Wizard	COX	
C6	*Paramecium multimicronucleatum*	cultured	chelex	COX	
		cultured	chelex	SSU rDNA	JN244001
E17	*Paramecium sp.*	creek water	Wizard	COX	
E18	*Paramecium tetraurelia*	cultured	chelex	SSU rDNA	JN244006
D6	*Strobilidium caudatum*	filtration	Wizard	SSU rDNA	JN244002
A2	*Tetrahymena sp.*	centr. creek	Wizard	SSU rDNA	JN243999
E6	*Tetrahymena sp.*	centr. creek	Wizard	SSU rDNA	JN244007
E14	unidentified ciliate	creek water	Wizard	SSU rDNA	JN244005
F9		centr. creek	Wizard	SSU rDNA	JN244014
F7	unidentified ciliate	centr. creek	Wizard	SSU rDNA	JN244013
F4	*Urocentrum turbo*	centr. creek	Wizard	SSU rDNA	JN244011

"Sampling Methods" and "DNA Isolation" for ciliates are described in the Materials and Methods. The COX gene or and SSU rDNA fragment was amplified, cloned, and sequenced. Ciliates were identified by comparison with published sequences using a BLAST search. "Sample" name indicates the collection sites referred to those indicated in Figure 1. The GenBank accession number is indicated for the SSU rDNA sequences.

campylum (X56532). The two differences are both single-base deletions in our sequence. One other clone from this site (C2) differed by one transition from the other four clones. Clones D2 and E10 were also identical to C1, C3, C4, and C5, while D1, D3, D4, and D5 only varied by only one or two pyrimidine transitions. Although E12 also matched most closely to *Colpidium campylum* (X56532), it showed considerable divergence from the others (621/639 bases, 97.2% pairwise identity with several deletions). DNA of all D site samples was isolated from filtered creek water samples using the Wizard method. DNA of E10 and E12 was isolated from centrifuged tuna trap water using the Wizard method.

4) C6 (submitted to GenBank as JN244001). This large ciliate was collected from a tuna trap and has been cultured on rye grass medium. DNA was isolated from cultured C6 ciliates using the chelex method. Three clones of its COX genes yielded slightly different sequences.

Two clones of C6's amplified COX DNA showed 99.9% pairwise identity (766/767 bases), and one clone showed 99.7% pairwise identity (766/768 bases, 1 gap) with a published *P. multimicronucleatum* sequence (AM072769). The presence of multiple sequences in this *P. multimicronucleatum* culture is covered in the Discussion. SSU rDNA was also sequenced from this ciliate and yielded a sequence that most closely matched a published sequence from an unknown species of *Paramecium* (99.7% pairwise identity, 636/638 bases), but the next closest match was with *P. multimicronucleatum* (97.0% pairwise identity, 624/643 bases, 10 gaps). This ciliate is freely available by contacting D. A. Johnson (djohnso2@samford.edu).

5) D6 (submitted to GenBank as JN244002). DNA of this ciliate was isolated from a filtered water sample using the Wizard method. D6 SSU rDNA showed 96.1% pairwise identity (589/613 bases, 3 gaps) with *Strobilidium caudatum* (AY143573).

6) E1, E2, E3, E4. DNA of these ciliates was isolated from centrifuged creek water samples using the Wizard method. E2's amplified COX DNA showed 99.1% pairwise identity (838/846 bases) with a published *Paramecium caudatum* sequence (FJ905142). E1, E3, and E4 all showed 98.9% pairwise identity (837/846) with FJ905142, but all three varied from each other by one substitution.

7) E6, E7, E8, E9 (E6 submitted to GenBank as JN244007). DNA of this ciliate was isolated from centrifuged creek water samples using the Wizard method. SSU rDNA from E6 , E7, and E8 yielded an identical SSU rDNA sequence that showed 100% pairwise identity (638/638 bases) with three different species of *Tetrahymena* (*T. lwoffi*, EF070250; *T. tropicalis*, EF070259 and EF070261; and *T. furgasoni*, EF070247) and also matched perfectly two unidentified *Tetrahymena* species (AY755629 and EF070265). One other clone, E9, differed by a single base transition from the E6, E7, and E8.

8) E11, E13 (E11 submitted to GenBank as JN244003). DNA of E11 and E13 was isolated from centrifuged tuna trap water using the Wizard method. This ciliate's amplified SSU rDNA was identical to published sequences for three different ciliates: *Hypotrichida sp.* (AF508777), *Pleurotricha lanceolata* (AF508768 and AF164128), and *Oxytricha sp.* (AF164684) (643/643 bases). It is listed as "unknown ciliate" in Table 1.

9) E14, E15, F9 (E14 and F9 submitted to GenBank as JN244005 and JN244014, respectively). E14 and E15 DNA was isolated from a creek water sample (no concentration of organisms by centrifugation or filtration) directly using the Wizard method. SSU rDNA of both most closely matched with unknown ciliates: 97.3% pairwise identity with AJ810076 and AY642718 (622/639 bases, 3 gaps). The closest match with a known ciliate was with *Glaucomides bromelicola* (AJ810077, 97.0% pairwise identity, 620/639 bases, 3 gaps). F9 DNA was isolated from a centrifuged creek water sample using the Wizard method. Its SSU rDNA was nearly identical to AY642718 (99.8% pairwise identity, 637/638 bases), and its closest match with a known ciliate was with *Glaucoma scintillans* (AJ511861, 98.4% pairwise identity, 629/639 bases, 3 gaps).

10) E17. E17 DNA was also isolated from a creek water sample (no concentration of organisms) using the Wizard method directly . E17 COX DNA showed 91.5% pairwise identity (773/845 bases, 6 gaps) with a published sequence for *Paramecium caudatum* (accession number FN424190). This ciliate appears to be a new species of *Paramecium* (see Discussion).

11) E18, E19, E20 (E18 submitted to GenBank as JN244006). This ciliate was cultured from tuna traps and has been maintained on rye grass medium. Its DNA was isolated by the chelex method, then its SSU rDNA amplified, cloned and sequenced. Two clones (E18, E19) had an identical 640 base sequence while E20 varied from these two by four separate transitions. E18

and E19's SSU rDNA had 99.8% pairwise identity (639/640 bases) with *Paramecium tetraurelia* (EF502045, AB252008, X03772, and AB252009).

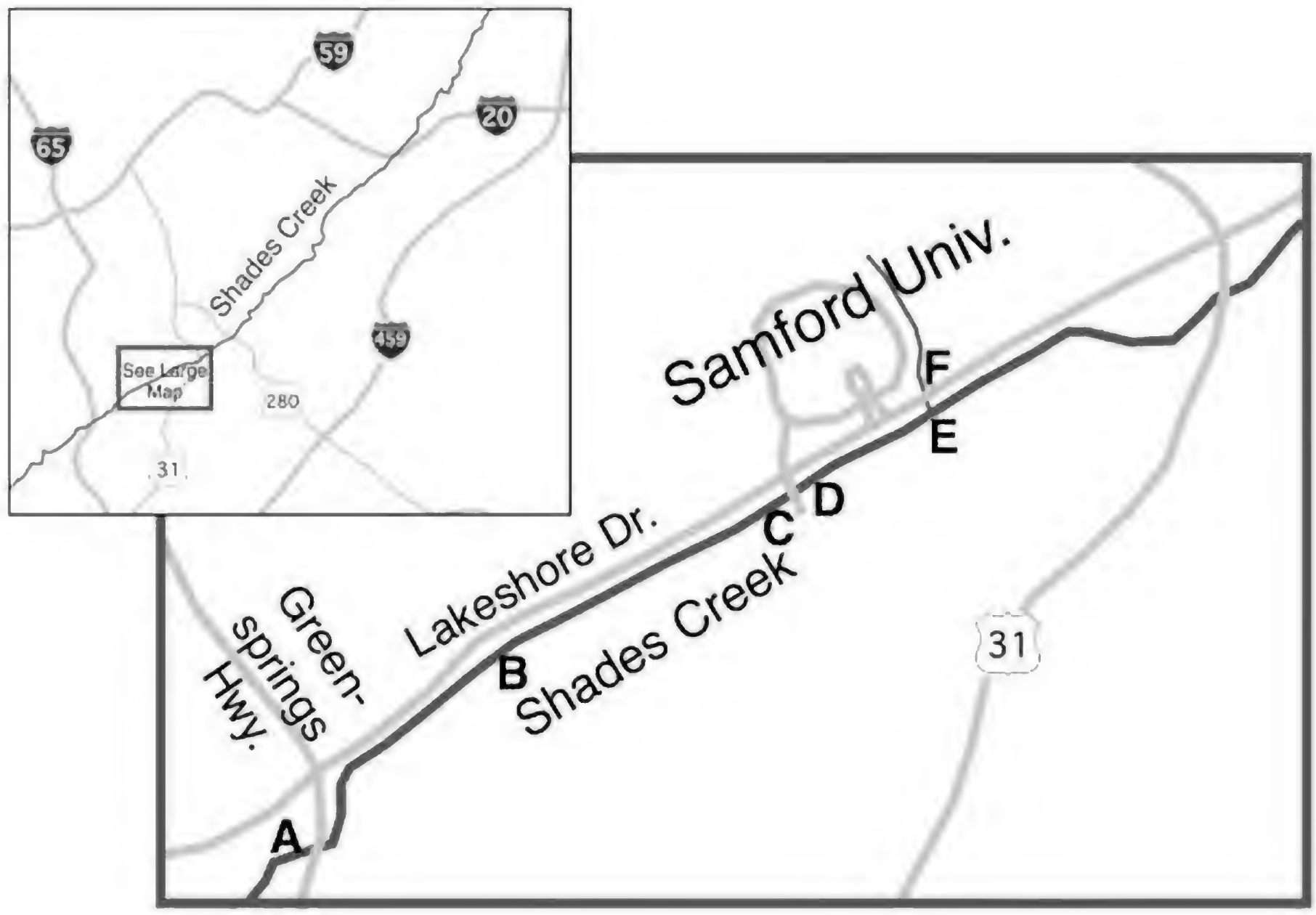

Figure 1. Ciliates collection sites.

Samples were collected at the lettered sites A through F in still or stagnant water. No sequence data was recovered from samples taken at site B. (See Materials and Methods for sampling methodology.)

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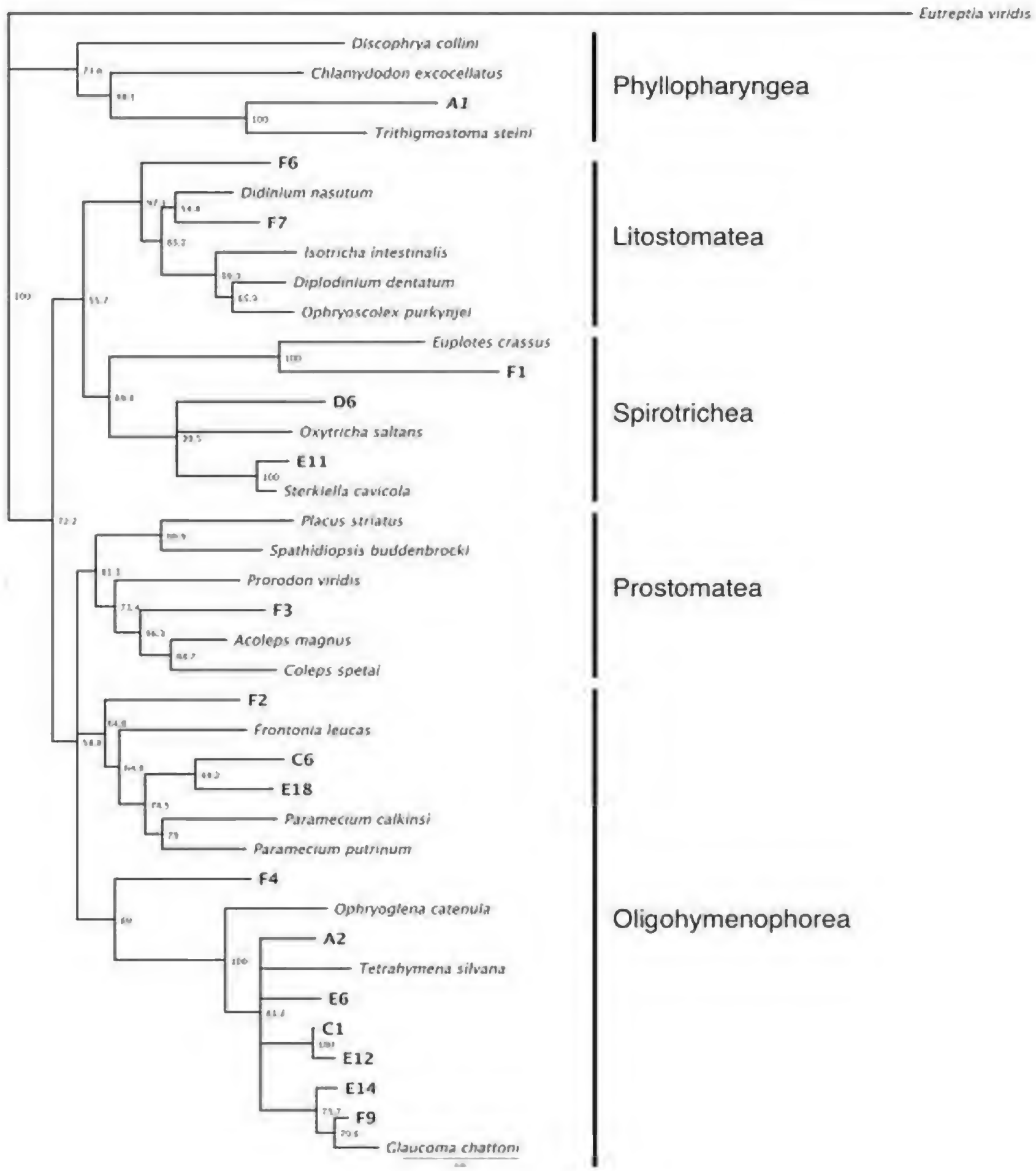

Figure 2. SSU rDNA phylogenetic tree of 15 Shades Creek ciliates.

This is a neighbor joining tree of relationships between 15 species of ciliates inferred from SSU rDNA sequences. The Tamura-Nei distance model was used, and numbers at the indices represent support values for 1000 bootstrap replicates. The scale bar corresponds to 5 changes per 100 nucleotides. The outgroup used was Eutreptia viridis (AJ532395, Eukaryota; Euglenozoa; Euglenida; Eutreptiales; Eutrptia. The class of each identified ciliate was inferred by the classification of the closest BLAST match. Class names are identified on the right.

12) F1 (submitted to GenBank as JN244008). DNA of this ciliate was isolated from centrifuged creek water samples using the Wizard method. Its SSU rDNA had 99.7% pairwise

identity (718/720 bases, 1 gap) with two published sequences of *Euplotes eurystomus* (EF193250 and AJ310491).

13) F2 (submitted to GenBank as JN244009). DNA of this ciliate was isolated from centrifuged creek water samples using the Wizard method. Its SSU rDNA had 99.7% pairwise identity (637/639 bases) with *Lembadion bullinum* (AF255358). Our sequence varied from the published sequence by 2 transitions.

14) F3 (submitted to GenBank as JN244010). DNA of this ciliate was isolated from centrifuged creek water samples using the Wizard method. Its SSU rDNA had 99.0% pairwise identity (624/643 bases) with *Levicoleps biwae* (AB354737). Our sequence varied from the published sequence by 12 single base substitutions and 7 single base insertions or deletions.

15) F4, F5, F8 (F4 submitted to GenBank as JN244011). DNA was isolated from centrifuged creek water samples using the Wizard method. F4's SSU rDNA had 97.3% pairwise identity (637/639 bases) with *Urocentrum turbo* (AF255357) while F5 and F8 were identical to AF255357 (641/641 bases).

16) F6 (submitted to GenBank as JN244012). DNA of this ciliate was isolated from centrifuged creek water samples using the Wizard method. Its SSU rDNA had 99.7% pairwise identity (571/573 bases) with *Loxophyllum spirellum* (GU574810). This cloned rDNA fragment was considerably shorter than that of our other sequenced rDNA fragments (573 bases versus around 640 bases for the rest). *Loxophyllum spirellum* has been identified from a marine source (Pan *et al.*, 2010), and the next eight closest matches on the NCBI BLAST site were also of marine origin. However, the tenth closest match (96.2% pairwise identity) is an uncultured ciliate identified in a freshwater constructed wetlands environment (Haentzsch *et al.*, 2010).

17) F7 (submitted to GenBank as JN244013). DNA of this ciliate was isolated from a centrifuged creek water sample using the Wizard method. SSU rDNA for F7 most closely matched with unknown ciliates: FN689996 and FN689997 (96.3% pairwise identity, 554/575 bases). The closest match with a known ciliate was with *Phialina salinarum* (EU242508, 96.0% pairwise identity, 620/639 bases, 3 gaps).

SSU rDNA Phylogenetic Tree

Fifteen unique ciliate sequences were isolated and compared using Geneious Pro software. Published sequences were included in the tree to better illustrate interclass relationships. *Eutreptia viridis* (AJ532395, Eukaryota; Euglenozoa; Euglenida; Eutreptiales; Eutrptia) was used as the outgroup.

As shown in Figure 3, the 15 ciliates fall into the classes Phyllopharyngea, Litostomatea, Spirotrichea, Prostomatea, and Oligohymenophorea. Bootstrap values for inner branches are variable and support tends to increase in intermediate and outer nodes. Classes Phyllopharyngea, Litostomatea, Spirotrichea, and Prostomatea appear to be monophyletic based on their appearance and their support values, which are all above 70%. The class Oligohymenophorea does not seem to be monophyletic based on its appearance. Other publications support the notion that Oligohymenophorea may not be monophyletic (Snoeyenbos-West *et al.*, 2004; Strüder-Kypke *et al.*, 2010).

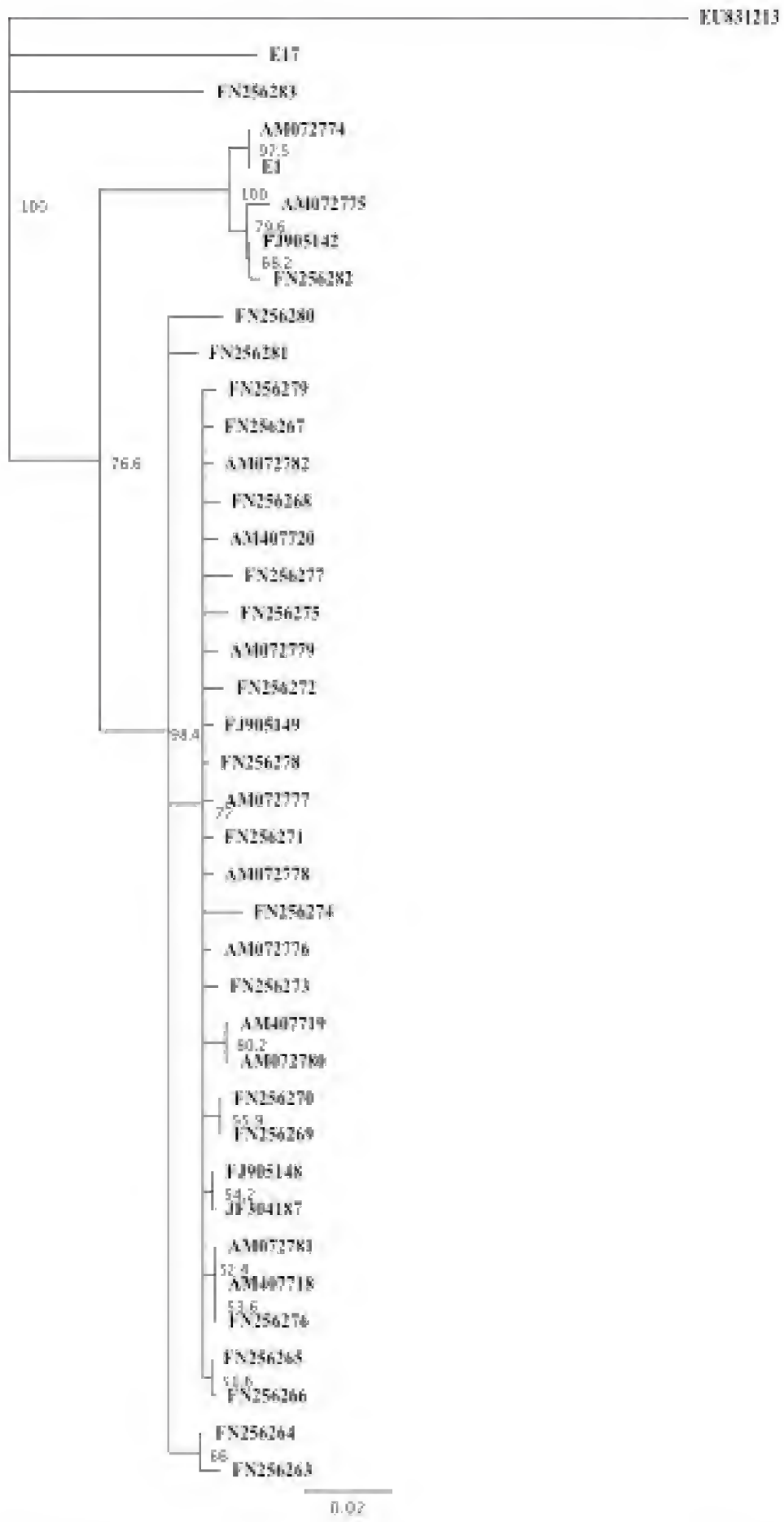

Figure 3. Phylogenetic tree of the E1 and E17 ciliate based on all published COX *P. caudatum* sequences.

This is a neighbor joining tree for all published Paramecium caudatum and species E17 and C6 inferred from cytochrome oxidase subunit I (COX) sequences. The Tamura-Nei distance model was used, and numbers at the indices represent support values for 1000 bootstrap replicates. The scale bar corresponds to 2 changes per 100 nucleotides

The two clades that Oligohymenophorea occupies appear to be more specific groupings of this class. The upper clade contains sequences from the order *Peniculida*. The lower clade contains sequences from the order *Hymenosyomatida*, with the exception of F4, which looks to be *Urocentrum turbo* based on its closest match from the BLAST search results (AF255357; 99.8% pairwise identity, 100% query coverage). If this sequence is *Urocentrum turbo,* it should fall within the *Peniculida* order. It should be noted that this same species failed to group within the

Oligohymenophorea class in a previous Ciliophora tree (Strüder-Kypke *et al.*, 2010). The most specific grouping within the lower Oligohymenophorea clade, with 83.2% support, appears to be the *Tetrahymenia* suborder based upon the taxonomy of the published sequences and all identifiable BLAST matches from our sequence data.

Deeper nodes, which denote class relationships, have low support values. However, there is high support (100%) for the classes Litostomatea, Spirotrichea, Prostomatea, and Oligohymenophorea being more closely related to one another than to Phyllopharyngea. Additionally, classes Litostomatea and Spirotrichea group together with low support (55.7%); however, this relationship is suggested by several publications (Riley *et al.*, 2001; Snoeyenbos-West *et al.*, 2004). Further, classes Prostomatea and Oligohymenophorea appear to be more closely related to one another than to the other classes, although the support for this grouping is once again low (58.8%). This relationship is also supported in several publications (Katz, 2001; Riley *et al.*, 2001; Snoeyenbos-West *et al.*, 2004).

COX DNA Phylogenetic Tree

A phylogenetic tree was built with Geneious Pro software comparing all GenBank published COX *P. caudatum* sequences with sequences E1 and E17 (Figure 3). *Miamiensis avidus* (EU831213, Eukaryota; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Scuticociliatia; Philasterida; Philasteridae; Miamiensis) was used as the outgroup. Tree topology for the COX DNA tree (Figure 3) is inferred to be reliable based upon the high level of branch support. All internal nodes have 70% support or above, and lower values are associated with terminal nodes. Additionally, it is important to note that the COX gene has the best resolution when comparing sequences at the genus or species level (Strüder-Kypke, 2010). Thus, we can be confident that the presented relationships do not distort actual evolutionary relationships since all sequences are within the same species, with the exception of E17, which was most closely related to *P. caudatum* and is within the *Paramecium* genus.

The branching pattern shows that species E17 and one *P. caudatum* species, FN256283, do not assemble with the major *P. caudatum* grouping (Figure 3). Each species has a long branch, indicating a high degree of divergence from the rest of *P. caudatum*. Within the main grouping of *P. caudatum*, there are two clades, both of which are well supported (100% and 98.4%). Terminal nodes seem to have lower support values, which can be accounted for by the lack of variation between most *P. caudatum* sequences, which include many identical genotypes.

DISCUSSION

The ciliate sequences observed in this preliminary survey of upper Shades Creek indicate that we have sampled 17 different ciliate species, based upon the closest BLAST matches. Table 1 indicates that of the strategies used in this report, concentration of creek water samples followed by DNA extraction by the Wizard method was most successful.

Due to the large amount of variation between ciliate species (Strüder-Kypke *et al.*, 2010), obtaining a phylogenetic tree with high interclass support values is unlikely, even when using the well-conserved SSU rDNA gene. Although our interclass support values were low, many relationships were identified that correlate with published Ciliophora phylogenetic relationships. Most importantly, our tree allows for the observation of the relative positioning of each of our isolates with published sequences, including several sequences whose closest BLAST match was an unidentified ciliate (E11, E14, F7, F9).

Sequences E14 and F9 grouped within the lower Oligohymenophorea clade with species that belong to the suborder *Tetrahymenia*, suggesting that they belong to this suborder. BLAST results suggest they may both be in the genus *Glaucoma*, which is supported by their grouping as shown in Figure 2. It is possible that the identities of these *Tetrahymenia* sequences could be reconciled by constructing a tree with published sequences from the suborder. Sequence F7 grouped within the Litostomatea clade, suggesting that it belongs within that class. Sequence E11 was identical to several published species that fall within the class Spirotrichea, as supported by the tree.

Our observation of ciliates present in tuna traps that were allowed to age for several days indicates that, not surprisingly, this "tuna-water medium" was selective. (It is also very pungent.) That is, after several days, certain ciliates, especially a very small one and a large one resembling *P. multimicronucleatum,* were predominant. In fact, the culture of *P. multimicronucleatum* that we have maintained since September of 2009 was derived from aged tuna water. This suggests that the strategy of aging tuna traps several days might be a successful method for isolating *P. multimicronucleatum.*

Ciliate DNA isolated from the E17 uncultured ciliate had only 91.5% pairwise identity with any published known species (*P. caudatum*, FN424190). It might be assumed from this BLAST match that E17 is *Paramecium* but not *P. caudatum*. A phylogenetic tree was built with Geneious Pro software comparing all GenBank published COX *P. caudatum sequences* (Figure 3). The COX DNA tree shows that all of these ciliates identified as *P. caudatum* group together, with the exception of species FN256283, and our isolate, E17.

We have little information on how species FN256283 came to be classified as *Paramecium caudatum*. After running a BLAST search on this species, we discovered that its closest match showed 93.2% pairwise identity and 100% query coverage with a published *P. caudatum* species (accession number: FN256270). The results from this BLAST search align with the phylogenetic relationships presented in Figure 3, suggesting that species FN256283 is more closely related to the *P. caudatum* species than sequence E17 due to its shorter branch length and higher BLAST score. However, FN256283 appears to be more distantly related to the *P. caudatum* species than any other published GenBank *P. caudatum* COX sequence.

Additionally, the COX DNA tree contains another species we isolated, E1. This uncultured ciliate showed 98.9% identity and 100% query coverage with a published *P. caudatum* sequence (accession number: FJ905142). E1 did fall within the main *P. caudatum* clade in the tree.

In this project, our goal was to identify as many unique ciliates as possible. The PCR and cloning methodology employed here has the disadvantage of producing multiple clones from a single organism. Since our primary objective was to determine which species are present in these

communities, this was not seen as a major problem. Also, single nucleotide differences between two strains could be due to errors during PCR amplification rather than naturally occurring diversity. However, our experience has been that Genewiz colony sequencing yields high quality, repeatable reads in the 600 to 700 bp range. Therefore, when major divergence was found, we assumed it to be significant. Consequently, we believe that the COX divergence seen in C6 indicates the presence of different haplotypes in this monoculture. This raises the possibility of heteroplasmy in *P. multimicronucleatum.*

Eight species described in this report were represented by only one sequenced isolate and one species by only two sequenced isolates, indicating that the ciliates identified here may be an incomplete picture of the ciliate communities present in our samples. We plan to continue this investigation of these communities and would expect the list of ciliate species present in Shades Creek to grow significantly. Future studies will include other techniques, including PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE)(van Hannen, 1998) and possibly metagenomic sequencing (using 454 pyrosequencing)(Margulies *et al.*, 2005), which promise to provide a more complete picture of the ciliate species comprising these freshwater communities.

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Fish Survey And Bioassessment Of Cox Creek In Lauderdale County, Alabama

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ABSTRACT

Cox Creek is a third order spring-fed tributary to Cypress Creek (Tennessee River drainage) in northwest Alabama, draining a portion of the city of Florence. This stream may have suffered the cumulative effects of urban watershed development over the last few decades, which could be reflected in the absence of historically documented species or in an Index of Biotic Integrity (IBI) assessment of the fish community. A fish survey and bioassessment of Cox Creek was conducted in 2010–2011 and IBI scores were calculated for eight localities that utilized the 30+2 sampling method of the Geological Survey of Alabama. Collections yielded a total of 37 species, with a mean of 20 species per collection, and records for seven species reported for the first time from Cox Creek. Including all historical records, the fish fauna of Cox Creek is 45 species and no previously documented species have declined in their presence in recent collections. Index of Biotic Integrity scores ranged from 38–50 out of 60 (fair to good/excellent) and showed a 6–12 point increase at downstream sites, suggesting a lack of cumulative biotic degradation of Cox Creek and the potential difficulty of using the IBI to assess smaller stream reaches. Continuing urban development within this watershed should be closely monitored to insure the aquatic biodiversity of this stream is not compromised, with strict maintenance of the existing riparian corridor.

Introduction

Alabama has at least 297 native fishes, which ranks among the richest fish faunas of any state (Boschung and Mayden, 2004). This species diversity is threatened by a variety of anthropogenic factors including urbanization, an influence found to contribute the most sediment (and other pollutants) as compared to agriculture and forestry practices (Jones and Holmes, 1985). Urbanization affects stream habitats and fishes in a number of other ways including increases in runoff volumes and rates, flooding events, and a reduction in base flow, often due to an increase in impervious surfaces such as parking lots (Wang et al., 2001). The amount of connected impervious surfaces shows a strong correlation with overall fish community health, as measured using the Index of Biotic Integrity (IBI; Karr, 1981; Wang et al., 2001; Miltner et al., 2004). Increasing urbanization has a measurable cumulative effect on the fauna, as stream fish communities show biological impairment at values as low as 5% impervious surface in the watershed, and can be irreparably damaged at values exceeding 27% (Schuler, 1994; Karr and Chu, 2000; Miltner et al., 2004). Riparian corridors serve as important buffers against sediment transport and streambank destabilization in urban areas, which often require the widest riparian corridors to be effective (Wang et al., 2001).

The IBI was originally developed by Karr (1981), and has been modified for application in many states (e.g. KIBI in Kentucky; Compton et al., 2003). The IBI uses fish communities as a bioindicator of stream health and attempts to reduce complex biological processes into a numerical index understandable to non-experts (Karr et al., 1986). The IBI traditionally contains three main categories and 12 metrics, each of which does not approximate (assigned a score of 1), somewhat approximates (3) or strongly approximates (5) the expected value at a reference stream for the region; the overall score may range from 12–60 (Karr et al., 1986; Compton et al., 2003). The Geological Survey of Alabama (GSA) has developed and calibrated the IBI for various watersheds across the state. In modifying the IBI for use in Alabama, correlation coefficients with habitat disturbances were used to select appropriate metrics that reflect alterations to fish communities due to anthropogenic factors (O'Neil and Shepard, 2007; O'Neil and Shepard, 2010).

In Alabama, five primary ichthyoregions are generally recognized, including the Tennessee Valley, which encompasses the northern one-fourth of the state (O'Neil and Shepard, 2010). Cox Creek lies within the Western Highland Rim Ecoregion of the Tennessee Valley, an area characterized by springs and caves, chert gravel streams, and high species diversity (Boschung and Mayden, 2004). The Highland Rim, which extends into Tennessee and Kentucky, has the most diverse fish fauna of any physiographic region of comparable size in North America (Etnier and Starnes, 1993). Cox Creek is a tributary to Cypress Creek, a watershed within Wayne County, Tennessee and Lauderdale County, Alabama, which drains approximately 650 km^2 before entering the impounded portion of the Tennessee River known as Pickwick Lake (Ceas and Page, 1995). Cypress Creek has a fish fauna of at least 83 species including one endemic, the crown darter *Etheostoma corona* Page and Ceas (Gooch, 1971; personal observation).

Cox Creek is a third order tributary to Cypress Creek and is located in the suburban area of Florence, Lauderdale County, Alabama. The watershed contains several intermittent springs and one permanent spring (King Spring), which add considerable volume to the stream, and contribute to an increase in channel width from 5 m near King Spring to over 25 m at the mouth of Cox Creek (personal observation). The watershed drains approximately 52 km^2 (O'Neil and Shepard, 2010) and continues to be developed for commercial and residential uses; these changes have resulted in additional impacts (e.g. road expansion) that have collectively altered watershed characteristics, including the amount of impervious surfaces (personal observation). O'Neil and Shepard (2010) calculated that 49% of the watershed was in urban land cover, with a population density of 342.13 people $\cdot$ km^{-2}. In overall human disturbance values, Cox Creek scored in the worst quarter percentile among Tennessee Valley streams sampled (O'Neil and Shepard, 2010). However, Cox Creek appears to have an intact riparian corridor along much of its length outside of six road crossings.

Collections made 1965–2009 were compiled from online database searches or personal communications with personnel from Auburn University, Cornell University, Geological Survey of Alabama, Illinois Natural History Survey, Tulane University, United States National Museum, and the University of Alabama Ichthyological Collection (FishNet 2, 2011). In total, previous researchers identified 38 species from 83 separate collections at just six localities. The main historical sampling locality was King (Buffler) Spring, from which 76 collections were made, likely in search of two spring-associated species: flame chub *Hemitremia flammea* (Jordan and Gilbert) and Tuscumbia darter *Etheostoma tuscumbia* Gilbert and Swain. A total of 2,715 and

2,494 specimens, respectively, are documented from collections made over the past 45 years, primarily by University of Alabama personnel (B.R. Kuhajda, personal communication, 25 January 2011).

The goals of this project were to provide an up-to-date fish faunal summary and an important baseline for monitoring future changes that may occur in the fish community of Cox Creek due to continued urban development.

MATERIALS AND METHODS

Sampling localities included sites from the headwaters to the mouth of Cox Creek. The 30+2 sampling method developed by the GSA was strictly followed at eight localities, including one site (Old Jackson Road) that was originally used in the IBI calibration sampling for the Tennessee Valley ichthyoregion (O'Neil and Shepard, 2010). The 30+2 method included 10 sampling efforts in each habitat of riffles, runs, and pools plus two shorelines of 46 m each for a total of 32 individual samples completed in a single-pass event. Fish sampling utilized a combination of backpack electrofishing (Smith-Root model LR–24) and seining (3.2 m x 1 m with 4.8 mm mesh) with a field crew of 2–3 individuals. Two collections that were made by a single individual using only backpack electrofishing and one collection that did not enumerate the catch were excluded from IBI analysis. In all collecting efforts, common species were identified, enumerated, and released. Vouchers for species were euthanized in clove oil and transferred to formalin in the field. After fixation, specimens were rinsed in water, and permanently stored in 70% ethanol for curation into the University of North Alabama–Florence Zoological collection. All data was recorded in Microsoft Excel and IBI values were calculated using a spreadsheet developed for the Tennessee Valley icthyoregion by GSA (O'Neil and Shepard, 2010).

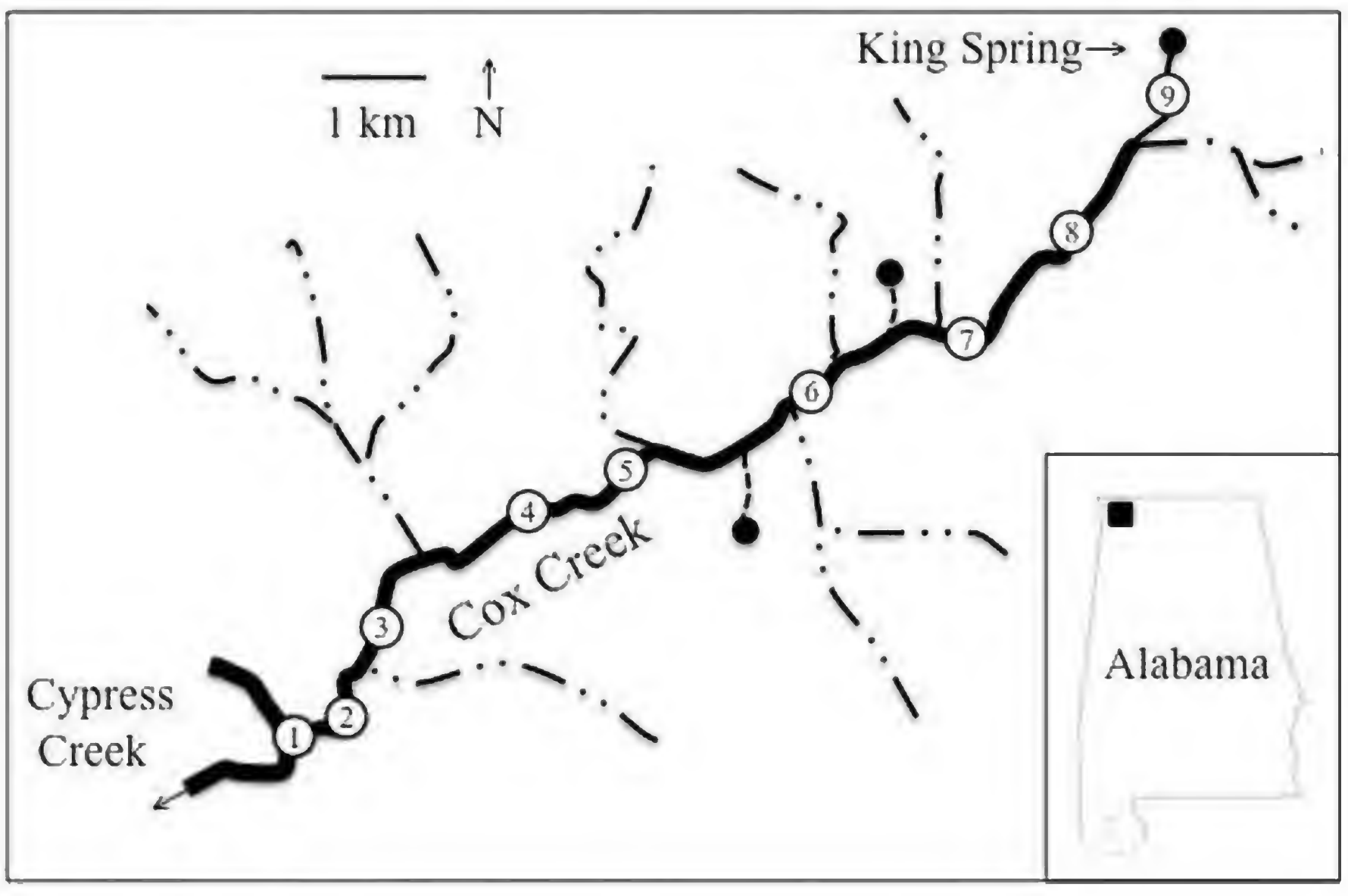

Fig. 1. Distribution of fish collection localities in Cox Creek, Lauderdale County, Alabama in 2010–11. Springs denoted by solid circles. Numbers correspond to sampling localities in Table I

RESULTS AND DISCUSSION

Eleven field collections were made at nine localities in 2010–2011 (Fig. 1; Table I). Localities four and seven were sampled separately two times, but only once following IBI protocols. From 2,029 individuals, 37 species were identified with a mean of 20 species per collection. Seven species not previously reported from Cox Creek included: river chub *Nocomis micropogon* (Cope), telescope shiner *Notropis telescopus* (Cope), golden redhorse *Moxostoma erythrurum* (Rafinesque), chain pickerel *Esox niger* Lesueur, smallmouth bass *Micropterus dolomieu* Lacepède, greenside darter *Etheostoma blennioides* (Rafinesque), and banded darter *Etheostoma zonale* (Cope). All species except *Esox niger* were regularly collected in fish community samples from the Tennessee Valley icthyoregion and are present in Cypress Creek (O'Neil and Shepard, 2010; personal observation). Based upon all collection records including historical data, the documented fish fauna of Cox Creek is 45 species, with 20 species considered common, 9 species limited, and 16 species sporadic (Table II).

Table I. Sampling localities, fish species diversity, and Index of Biotic Integrity values for Cox Creek, Lauderdale County, Alabama in 2010–11. Localities correspond to numbered sites in Figure 1.

Locality	GPS Coordinates	Species Diversity	Drainage area (km^2)	IBI score
1. Cox Creek just above mouth	34.828266, -87.701004	25	52	50
2. Cox Creek at Old Jackson Road	34.829019, -87.698595	24	43	48
3. Cox Creek 1 km above Jackson Road	34.834549, -87.696422	25	39	50
4. Cox Creek at Highway 157[a]	34.838041, -87.687952	21	34	50
5. Cox Creek at Cox Creek Parkway	34.838904, -87.684669	19	31	48
6. Cox Creek at Chisholm Road	34.843016, -87.674128	19	23	42
7. Cox Creek at Helton Drive[a]	34.845613, -87.666419	17	18	38
8. Cox Creek at Mars Hill Road	34.849974, -87.660921	14	13	40
9. Spring Run below King Spring[b]	34.855965, -87.653877	5	n/a	n/a

Notes: [a] sampled separately two times, but only once following IBI protocols, [b] not sampled following IBI protocols

A comparison of average species richness in historical collections (13 species) versus this study (20 species) suggests one or more of the following: historical collections involved less sampling effort, there has been no measurable decrease in species diversity in Cox Creek, or historical sampling targeted on rare species and fewer habitat types. Since many samples were made at or near King Spring (presumably to collect *Hemitremia flammea* or *Etheostoma tuscumbia*), the latter seems likely for most historical collections. Based upon the six historical

Table II. Fish species list for Cox Creek, Lauderdale County, Alabama. Species are categorized as common (C), limited (L) or sporadic (S) based upon the total number of records in historical and recent collections through 2011.

Scientific name	Common name	Occurrence
Ichthyomyzon castaneus	chestnut lamprey	S
Ichthyomyzon gagei	southern brook lamprey	S
Lampetra aepyptera	least brook lamprey	S
Campostoma oligolepis	largescale stoneroller	C
Clinostomus funduloides	rosyside dace	C
Cyprinella spiloptera	spotfin shiner	S
Cyprinella whipplei	steelcolor shiner	S
Hemitremia flammea	flame chub	C[a]
Hybopsis amblops	bigeye chub	L
Luxilus chrysocephalus	striped shiner	C
Luxilus coccogenis	warpaint shiner	L
Lythrurus fasciolaris	scarlet shiner	C
Nocomis micropogon	river chub	S
Notropis telescopus	telescope shiner	L
Pimephales notatus	bluntnose minnow	S
Rhinichthys atratulus	blacknose dace	C
Semotilus atromaculatus	creek chub	C
Catostomus commersoni	white sucker	S
Hypentelium nigricans	northern hog sucker	C
Moxostoma duquesnei	black redhorse	S
Moxostoma erythrurum	golden redhorse	L
Ameiurus natalis	yellow bullhead	S
Esox niger	chain pickerel	S
Fundulus catenatus	northern studfish	L
Fundulus olivaceus	blackspotted topminnow	C
Gambusia affinis	western mosquitofish	C
Cottus carolinae	banded sculpin	C
Ambloplites rupestris	rock bass	C
Lepomis cyanellus	green sunfish	C
Lepomis macrochirus	bluegill	C
Lepomis megalotis	longear sunfish	C
Micropterus dolomieu	smallmouth bass	L
Micropterus salmoides	largemouth bass	L
Etheostoma blennioides	greenside darter	L
Etheostoma blennius	blenny darter	S
Etheostoma caeruleum	rainbow darter	L

Etheostoma corona	crown darter	C
Etheostoma duryi	black darter	C
Etheostom flabellare	fantail darter	C
Etheostoma rufilineatum	redline darter	S
Etheostoma tennesseense	Tennessee darter	C
Etheostoma tuscumbia	Tuscumbia darter	C[a]
Etheostoma zonale	banded darter	S
Percina caprodes	logperch	S
Aplodinotus grunniens	freshwater drum	S

Notes: [a] Common in King Spring only

records from localities other than King Spring, there also appears to be no measurable increase in tolerant species, as recent collections do not contain a greater percentage of individuals categorized as pollution tolerant (O'Neil and Shepard, 2010). Tolerant species made up 17–32% of the individuals collected, with a resulting IBI score of 3 at all sites in collections made for this study (Table III).

Table III. Index of Biotic Integrity (IBI) scores for Cox Creek, Lauderdale County, Alabama using metrics developed for the Tennessee Valley ichthyoregion (O'Neil and Shepard, 2010). Sampling localities correspond to sites listed in Table I.

	Sampling locality							
IBI metrics	1	2	3	4	5	6	7	8
1. Total native species	5	5	5	5	3	5	3	3
2. Number shiner species	3	3	3	5	5	3	3	1
3. Number sucker species	3	3	3	3	3	3	3	3
4. Number of darter + madtom species	5	5	5	5	3	5	3	5
5. Percent of tolerant species	3	3	3	3	3	3	3	3
6. Percent *Lepomis*	5	5	5	5	5	5	5	5
7. Percent omnivores	5	5	5	3	5	1	3	3
8. Percent invertivores	5	5	5	5	5	5	3	5
9. Percent top carnivores	3	5	5	5	3	1	1	1
10. Percent DELT + hybrids	5	1	5	5	5	5	5	5
11. Number of lithophilic spawners	5	5	5	5	5	5	5	5
12. Number of intolerant species	3	3	1	1	3	1	1	1
Total	**50**	**48**	**50**	**50**	**48**	**42**	**38**	**40**

At eight localities that strictly followed prescribed GSA 30+2 sampling protocols, IBI values ranged from 38–50 (fair to good/excellent) with a mean of 46 (Table III). Overall, IBI scores

were always lower at upstream sites as compared to localities in the lower one-half of the watershed (38–42 versus 48–50; Table I); these sites contained less species diversity, a factor potentially impacting four IBI metrics that measure species richness and composition: number of native species, number of shiner species, number of sucker species, number of darter + madtom species (Karr et al., 1986; O'Neil and Shepard, 2010). Although scoring of these metrics is adjusted based upon drainage area (with fewer species expected in smaller streams), the ability to statistically discriminate among undisturbed versus disturbed habitats in smaller watersheds like Cox Creek is difficult (O'Neil and Shepard, 2010). Assessing sites that have very small (or large) drainage areas may be the most prominent limitation of the IBI (Compton et al., 2003).

The metrics in this study that most often strongly approximated the expected value at a reference stream included the number of lithophilic spawners, percentage *Lepomis*, and percentage invertivores. These three metrics are indicative of clean gravel for reproduction, stable trophic composition, and a low percentage of species that tolerate stream impairment, respectively (O'Neil and Shepard, 2010).

The two metrics that most often did not approximate conditions at a reference stream were the number of intolerant species and the percentage of top carnivores. Most species categorized as intolerant in Alabama are in the family Cyprinidae ("minnows"), but Cypress Creek (including Cox Creek) has moderate minnow species diversity among Tennessee Valley streams (Boschung and Mayden, 2004). Top carnivores may be difficult to collect even when present, but high quality streams with a well-structured fish community usually contain top carnivores. This metric can help segregate the very high quality, excellent sites from the good to very good sites (O'Neil and Shepard, 2010).

In comparing a collection made by GSA in 2009 with a collection from this study at the same locality (site two), the IBI differed by ten points (38 versus 48), although the sampling effort (32 efforts each) and total number of individuals captured was comparable (265 and 229, respectively). Based on individual metric scores, the disparity is primarily accounted for by a difference in species diversity (20 versus 24) and percent top carnivores (1% versus 5%), which altered the overall score from fair to good (O'Neil and Shepard, 2010).

Since few historical records outside of King Spring are available to compare the historic fish community composition of Cox Creek, with no known records prior to 1965, it is difficult to determine how the composition of the fish fauna has been altered by anthropogenic factors. When compared with other streams in the Tennessee Valley ichthyoregion, no obvious measurable changes have yet occurred. However, the fish community may be irreparably damaged as urban development continues in the watershed (Karr and Chu, 2000; Miltner et al., 2004). To document any changes in the fish community, annual sampling should be conducted over the next several years (and decades) as urbanization continues. As an aid to preserve the biotic integrity of Cox Creek, it is recommended that the current riparian corridor be strictly maintained, with no impervious surfaces established within a minimum 50 m stream buffer (Wang et al., 2001), and developers be required to use best management practices to minimize erosion throughout the watershed.

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THE MORAL RESPONSIBILITY OF SCIENTISTS

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ABSTRACT

Research scientists are commonly the first to become aware of new discoveries that may harm other human beings. These researchers will also be best situated to determine what harms these discoveries may bring and how they may do so. Therefore, these scientists should accept the moral obligation to prevent others from being harmed as a result of their discoveries. However, individual scientists are hampered by several factors: There are no commonly accepted guidelines to determine which discoveries are likely to cause significant harm to others and no authority to guide them on such matters. Also, they can do no more than alert others to dangers. They cannot take additional action to prevent harm from coming to others. Hence, alerts from scientists must be the first step to protect human beings and not the last. Relevant institutions, whether governmental, academic, or commercial have the additional obligations of devising workable guidelines, establishing consulting agencies, and putting means in place to protect the public from harm. Without these additional measures, the actions of individual scientists must be ineffectual.

INTRODUCTION

"When you see something that is technically sweet, you go ahead and do it and you argue about what to do with it only after you have had your technical success. That is the way it was with the atomic bomb" (Robert Oppenheimer, quoted in Bernstein, 2004).

Roald Hoffmann is a prominent member of the small roster of Nobel laureate chemists who are also serious playwrights. His recent play *Should've* was written to raise an issue and prompt a discussion. Hoffmann wants people (scientists, in particular) to ask whether individual researchers should consider themselves morally accountable for the consequences of their discoveries. But, because he is a playwright as well as a scientist, he wants his audience to *feel* this difficulty before they reflect on it, so his plot is carefully designed to elicit this feeling.

Though he has committed suicide shortly before the first scene opens, Friedrick Wertheim is the central focus of the play. Like Roald Hoffmann, he was a refugee from Nazi persecution and an accomplished chemist. As chemists will, Wertheim chanced upon a way to quickly and easily synthesize a substance that intrigued him. From his perspective, it was of only passing interest that the substance, saxitoxin, is a deadly agent that can paralyze or kill those who ingest it. As Robert Oppenheimer understood, Wertheim saw the way to a neat synthesis and developed it without giving the matter further consideration. Sometime later, he discovered that an Uzbek warlord employed his technique to generate a large quantity of saxitoxin and then used it to poison hundreds of people. Quite understandably, Wertheim was devastated by this

information. Shortly thereafter, he committed suicide and left a note in which he stated that he did so because he believed himself responsible for the mass death. Nonetheless, Hoffmann is sufficiently attentive to the ambiguities of human life and the craft of play writing to salt in other distressing factors, including a bitter argument between Wertheim and his daughter and news that his parents had been saved from death camps by a Nazi official. In combination, these additional factors allow the possibility that Wertheim's suicide was motivated by an array of forces and not by his chemist's guilt alone. In this way, Hoffmann neatly calls into question both Wertheim's motives for suicide and the moral accountability of the scientist for the consequences of research. He does not want to settle these matters for us but leave them open for our consideration.

Should've raises several questions. First, do scientists bear moral responsibility for the consequences of their discoveries? Second, if they do have such a responsibility, what can they do to fulfill it? Finally, are the efforts and knowledge of individual scientists *sufficient* to address any dangers resulting from scientific discovery?

THE MORAL BURDEN OF INDIVIDUAL SCIENTISTS

Friedrick Wertheim

As Hoffmann portrays him, Professor Wertheim held firm moral principles and was greatly distressed by the world's evil. Nonetheless, his rectitude did not move him to consider the possibility that his research would yield tragic consequences. Given his character and his dismay at the evil use of his synthesis, we may presume that he would have been appalled by the prospect of contributing to hundreds of human deaths and would have been eager to prevent them. He certainly had no desire to kill people, and he played no direct role in the atrocity. So, Wertheim's *intentions* and *character* are not in question.

Though Wertheim was understandably distraught over the evil employment of his method of synthesis, it is possible he had absolutely no *moral* responsibility for its misuse. He devised the first link in a chain of events that resulted in mass death. However, it is not necessarily true that his causal contribution is morally relevant. He neither intended nor foresaw the deaths that resulted from his discovery. Simply playing a causal role in producing an event is not sufficient to incur moral guilt. Some morally relevant defect of conduct or intent must also be present. It is nonetheless possible that Wertheim was guilty of carelessness or recklessness. Sometimes people are morally blamed for thoughtlessly creating conditions that may cause harm. Others may become aware of dangerous conditions, such as a weakened staircase or defective electrical wiring, but are blamed either for failing to correct the hazards or give proper warning to others.

So, did Wertheim fail to exercise a moral duty to protect others from harm? It is likely that Wertheim was following the usual scientist's maxim that all scientific knowledge is good. And, as Roald Hoffmann puts it, "There are no bad molecules" (Hoffmann, 1995, p. 139). Scientists, chemists in particular, are accustomed to working with dangerous substances. Such work is a normal part of their jobs. They are expected to take measures to prevent themselves and others from being directly harmed by their work. However, they are not customarily thought accountable for all possible uses of their discoveries. So, Wertheim's activity was no different from that of a typical, conscientious chemist and did not fall short of the standard of care expected of members of his profession. In like fashion, though guns and knives are commonly

used to cause grievous harm, weapons manufacturers are not typically held morally accountable for those tragic outcomes. In consequence, it appears that Wertheim's conduct was not morally faulty. Like a careful and conscientious driver who injures a pedestrian who darts carelessly into a street, Wertheim suffered the remorse of a morally blameless agent. Nonetheless, any morally blameless agent of death is likely to wish to prevent similar deaths in like circumstances. What is more, the public at large is likely to share that wish. All may seek to use the occasion of tragedy to consider measures that may prevent its repetition in the future.

In this light, it is possible that Hoffmann wishes to pose a different question: *Should* scientists henceforth shoulder the responsibility of asking whether their discoveries are apt to cause harm? Perhaps humanity would be far safer if scientists began to consider the potential uses and misuses of their discoveries. As it happens, we have striking examples of scientists who addressed these questions carefully and conscientiously.

Ian Ramshaw and Ronald Jackson

Ian Ramshaw and Ronald Jackson had the best of intentions. They sought an effective and humane way to control the populations of field mice that were major agricultural pests in their native Australia. Their plan was to alter mouse pox virus so it would make the mice sterile. In typical cases, mouse pox makes infected animals only mildly sick. Furthermore, in the usual experience of researchers, modifying a virus makes it *less* potent (BBC World Service, 2001). So, they infected the mouse pox virus with Interleukin-4, a naturally occurring molecule that boosts antibody production. Ramshaw and Jackson hoped that the additional antibodies would attack mouse eggs, thus sterilizing the mice (Nowak, 2001). Using standard genetic engineering techniques, they introduced Interleukin-4 into the virus. To test the result, they then infected groups of mice that had not been vaccinated against mouse pox and groups that had been vaccinated. Ramshaw and Jackson were surprised to discover that the altered mouse pox killed unvaccinated mice. But, they were both startled and dismayed to discover that many of the vaccinated mice also died. Up to that point, no known method had ever altered a virus to overcome the defenses of an organism that had been vaccinated against it (Selgelid and Weir, 2010).

Humans are not affected by mouse pox. However, mouse pox is a close kin of smallpox, a deadly killer of human beings. Because smallpox was eliminated in the 1970s, medical practitioners have limited experience dealing with it, and the world's supply of vaccine is small. Ronald Jackson made the obvious inference quickly: "It would be safe to assume that if some idiot did put human IL-4 into human smallpox, they'd [sic] increase the lethality quite dramatically" (Nowak, 2001).

Drama aside, Jackson and Ramshaw's deliberations highlight the same issue that plagued Frederick Wertheim: Do scientists have any moral obligations other than their professional responsibility to discover and transmit scientific truth? It seemed obvious to Jackson and Ramshaw that they did, indeed, have such obligations. The very considerable danger their results implied made this an obvious conclusion. Their obligation followed from their understanding of the danger their experimental results revealed. It did not result from their causal role in creating the altered virus or from their status as scientists. But, if they do have an obligation, what precisely is it, and is it the same for all scientists?

Saxitoxin and small pox

Jackson and Ramshaw's circumstances and concerns were vastly different from Wertheim's. The Australians were laboring in a world keenly attuned to the dangers of lethal biological agents. Several nations were constructing institutional means to address these perils. Also, of course, smallpox is a notoriously deadly biological agent, sufficiently notorious that the world took considerable pains to eradicate it in the 1970's. So, the two researchers found themselves in a climate fully alert to the dangers posed by smallpox and its potential use as an instrument of mass death. Nearly any educated person would have been alarmed by the prospect of a revived and easily produced smallpox.

Wertheim's circumstances were very different. Though saxitoxins are largely unknown to the general public, a number of officials consider them to be a public health menace. In nature, saxitoxins are produced by a variety of algae that are eaten by shellfish. If humans eat these shellfish in turn, they may be paralyzed and perhaps die (RaLonde, 1996, p. 1). However, the international Chemical Weapons Convention lists saxitoxins as a Schedule 1 Chemical. It defines such chemicals as substances that "Have little use for purposes other than those prohibited under the CWC (Chemical Weapons Convention Bulletin)." Nonetheless, it is unlikely that either Wertheim or other chemists were specifically alerted to the possible dangers of synthesizing saxitoxins. As noted earlier, there is little reason to conclude that Wertheim would have considered his synthesis an agent of mass death. Many other, more familiar agents are at hand for such purposes. In retrospect, it would obviously have been better if he had made inquiries about saxitoxins, but he would have had little reason to be particularly concerned by them. That said, it is unlikely Wertheim, left to his own devices, would have concluded that saxitoxins pose a danger to humanity comparable to smallpox. He would need guidance from elsewhere to justify such a conclusion.

Hence, if scientific researchers such as Wertheim are expected to be aware of the potential dangers of biological agents unfamiliar to the general public, such as saxitoxins, the scientists must have efficient access to the information they need. It is likely that nothing more elaborate than a searchable website would suffice. With a website in place, a quick check would have given Wertheim everything he needed to know about the dangers associated with saxitoxins. However, a searchable website is of little consequence if scientists lack the habit of using it whenever they consider working with a substance. A concerted effort would be needed to sensitize them to the dangers of such agents, and researchers would need periodic reminders of the potential dangers of the substances they use. In other words, the culture of scientific investigation would have to shift away from the reflexive faith that all knowledge is good to an understanding that the potential goodness of some types of knowledge may be outweighed by the perils they pose.

MODES OF RESPONSE

With their data in hand, Ramshaw and Jackson had to decide what to do next. Though the results of their experiment became clear well before 9-11 and the anthrax attacks in the United States, the scientific community was already attuned to the possibility of bioterror attacks. Nonetheless, there were no guidelines or advisory committees to assist scientists in their deliberations (Selgelid and Weir, 2010). And so, the two researchers were left to their own devices. They did check with the Australian Department of Defense, which offered no objection

to publishing their results (Nowak, 2001). Obviously, two less alert or less conscientious researchers may well have failed to make the inferences they made or to deliberate as they did. They acted entirely on their own initiative. After considerable reflection, Ramshaw and Jackson decided that their best course would be to publish the results of their work rather than suppress them. As Jackson explained, they reasoned, "We wanted to warn the general population that this potentially dangerous technology is available. We wanted to make it clear to the scientific community that they should be careful, that it is not too difficult to create severe organisms" (Nowak, 2001). In consequence, their report was published in the *Journal of Virology* in February of 2001 (Jackson, et al., 2001). Their decision to warn is well grounded provided we accept two assumptions: First, given the present state of science, it is likely that someone would stumble on this technique sooner or later whether or not Jackson and Ramshaw published their results. Second, someone would eventually decide to use this technique to cause harm to other human beings.

The Australian mousepox case highlights an important point: Other than warning of the dangers their discovery posed, there was little Jackson and Ramshaw could have done to make their concerns effective. Their warning succeeded only because the rest of humanity was sensitized to the dangers of a rejuvenated smallpox virus. Of course, they realized the consequences of their manipulation only after their experiment. They had no idea it might have the result it did. But, at that juncture, they still had the option of refusing to publish their work and attempting to keep it secret. However, as noted earlier, they assumed that someone else would inevitably duplicate their experiment or something like it. If so, refusal to publish or an attempt to maintain secrecy would have been futile. Further, they would also have been irresponsible if, by keeping their information secret, they deprived humanity of a warning that might have allowed it to address the dangers their experiment uncovered. So, they published their results and warned humanity. In this case, their warning was enormously successful. News of their experimental results and expressions of concern over the implications of their work quickly appeared in major publications across the globe (BBC, 2001; Radford, 2001; Nowak, 2001; Selgelid and Weir, 2010; Fraser and Dando, 2001; Harris, 2001; Pollak, 2001; Wade, 2003). Among other consequences, Ramshaw and Jackson's warning played a significant role in motivating a United States report on the hazards of biological research and construction of guidelines to address those hazards (National Research Council, 2004; Wade, 2003).

Once again, Wertheim's circumstances differed. As Hoffmann described it, Wertheim's method of synthesizing saxitoxins was extremely simple, the sort of thing that is obvious with hindsight (Hoffmann, 2006). If that is correct, and if it is also reasonable to assume that other researchers would eventually have discovered Wertheim's method of synthesis, he could not have been expected to protect humanity from danger by refusing to publish his results. Hence, like Jackson and Ramshaw, his only recourse would have been to give warning. That said, it is unlikely that the world would have been as electrified by a warning about saxitoxins as it was by the warning about smallpox. Apart from a small body of specialists, the world was attuned to the dangers of smallpox virus in a way it was not attuned to the possible dangers posed by saxitoxins. A warning must be the first step in an effective response, but it cannot be the last. If that is correct, would Wertheim have been obligated to attempt to make his warning successful? If so, his obligation would not have ended with a warning. However, most chemists have neither the skills nor resources that would allow them to transform their warnings into an effective

response. As it happens, Jackson and Ramshaw, being biologists, lacked these resources as well. Their warning was successful only because humanity was prepared to receive it and respond to it. Scientists have neither the power nor authority to control or halt social processes.

ARE THE ACTS OF INDIVIDUAL SCIENTISTS *SUFFICIENT* TO PROTECT OTHERS?

In June of 1945, the atomic researchers of the Manhattan Project neared completion of their work on the atom bomb. To their horror, they then realized that once their work was completed, they would have no control over the weapon they had devised. They had been eager to construct the atomic bomb in order to counter the threat they believed Nazi Germany posed. But, with the war in Europe at an end, they realized the device might be used against Japan. In consequence, they addressed a letter to President Truman urging that the bomb not be used and providing a careful analysis to support their contention that dropping the bomb would result in greater danger to humanity than refraining (Manhattan Project "Metallurgical Laboratory," 1945). Of course, their pleas fell on deaf ears. Though their circumstances were more dramatic than those of contemporary scientists and they were an extraordinarily distinguished group, many others will share their stunned recognition that individual scientists are unable to control the ways in which their discoveries are used. They understood, as did Jackson and Ramshaw, that they could only give warning. Wertheim, too, could only have given a warning. Nonetheless, his warning would likely have been ineffective. If that is correct, and, if it is reasonable to assume that humanity is owed warnings by those who are aware of dangers to human life, then other institutions, such as professional associations, businesses, academic institutions, and governments will have to shoulder the burden of protecting the public from potential harms resulting from scientific discovery.

CONCLUSION

The thing to keep in mind is that scientists are human beings. They have special knowledge and skills and may focus more intently than other mortals, but they remain human all the same. In part, this implies that their decisions and sense of responsibility are shaped by their environments in much the same way that cultural environments shape the values and perspectives of other human beings. At present, scientists are not trained to consider the potential ill consequences of their discoveries, and they typically are not expected to do so. When they give thought to such matters, they are inclined to think of all scientific knowledge as good or to focus on the hoped-for positive consequences of their research and dismiss the potential dangers. Apart from a few domains of science, they are not inclined to believe that the results of their research endanger humanity.

Frederick Wertheim lived and worked in such circumstances. Though Wertheim was extraordinarily sensitive and morally engaged in his personal life, those concerns did not carry over to his activity as a chemist. He was well aware that saxitoxins can be deadly to human beings, and that danger may have been part of the attraction for him. But, it would likely never have occurred to him to consider saxitoxins a potential instrument of mass murder. Unlike smallpox, they are not closely linked to horrible death in the public mind or in the minds of

scientists. Moreover, even extraordinarily deadly toxins often have valuable roles to play in medical research. In consequence, Professor Wertheim had no particular reason to be concerned about the deadly compound in his laboratory. Even had he considered its potential dangers, he still would have had no special reason to think of it as an instrument of mass death.

And so Wertheim found himself in a moral no-man's-land. The values and obligations he had come to accept as a result of his professional training and experience were no longer adequate to the conditions he faced. Yet, he had no others to replace them. The difficulty is that neither he nor any other individual is capable of formulating a set of moral guidelines to address the ever-changing circumstances of science. That is a work for the community of scientists as a whole and also of the public at large, who are greatly affected by the work scientists perform.

Even with a new set of moral guideposts, Wertheim, acting alone, could not have prevented any of the dangers to humanity resulting from his scientific discovery. He could, at most, raise an alarm. If no one else was receptive to his alarm and no institutions equipped to act on it, his act would have been futile. Hence, the activity of a solitary scientist can be only the beginning of a sequence of events that addresses the dangers of scientific discovery. At minimum, there should be some simple and convenient means for researchers such as Wertheim to check to determine whether substances of interest to them are likely to be dangerous to humanity. Wertheim would likely have welcomed such a resource. However, many researchers will be too harried or unconcerned. Hence, it would also be necessary to take measures to instill a culture of moral concern in the members of the scientific community. But, yet again, individual scientists can give warnings and that is all. So, institutional resources would need to be constructed to receive such warnings, evaluate them, and, if necessary, devise appropriate modes of response. Unfortunately, this more more paperwork, more cumbersome bureaucracy, and more restrictions on scientific activity. Though scientists may rightly grumble over these entanglements, they are part of a necessary response to the powerful results of scientific activity, the enormous amounts of money that may follow scientific discovery, and the diminishing boundary between pure and applied research.

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AGENDA
ALABAMA ACADEMY OF SCIENCE
FALL 2011 EXECUTIVE COMMITTEE MEETING
SAMFORD UNIVERSITY

SATURDAY, October 8, 2011, William Self Propst Hall, Room 033
beginning at 8:00 AM

A. Call to order and approval of minutes of the Spring 2011 Executive Committee Meeting [Minutes on the Academy website]

B. Officer Reports.....

1. Board of Trustees — Ken Marion
2. President — Mickie Powell
3. President -Elect — Ronald Hunsinger
4. Second Vice President — Emanuel Waddell
5. Secretary — Janie Gregg
6. Treasurer — Bettina Riley
7. Journal Editor — Safaa Al-Hamdani
8. Counselor to AJAS — Catherine Shields
9. Science Fair Coordinator — Virginia Vilardi
10. Science Olympiad Coordinator — Jane Nall
11. Counselor to AAAS — Steve Watts
12. Section Officers

I.	Biological Sciences	Ketia Shumaker
II.	Chemistry	Emanuel Waddell
III.	Physics & Mathematics	Manmohan Aggarwal
IV.	Engineering & Computer Science	Qichao Liu
V.	Social Sciences	Richard Hudiburg
VI.	Anthropology	Harry Holstein
VII.	Science Education	Janet Gaston

VIII.	Industry, Environmental, and Earth Science	Janie Gregg
IX.	Health Sciences	Vacant
X.	Bioethics & History/Philosophy of Science	Lewis Barker

13. Executive Officer — Larry Krannich

C. Committee Reports.....

1.	Local Arrangements	Prakash Sharma
2.	Finance	Ken Marion
3.	Membership	Vacant
4.	Research	George Cline
5.	Long-Range Planning	Adrian Ludwick
6.	Auditing, Senior Academy	Robert Angus
7.	Auditing, Junior Academy	Catherine Shields
8.	Editorial Board & Associate Journal Editors	Thane Wibbels
9.	Place and Date of Meeting	Jimmy Triplett
10.	Public Relations	Vacant
11.	Archives	Troy Best
12.	Science and Public Policy	Scott Brande
13.	Gardner Award and AAS Fellows	Prakash Sharma
14.	Carmichael Award	Richard Hudiburg
15.	Resolutions	Ketina Shumaker
16.	Nominating Committee	Emmanuel Waddell
17.	Mason Scholarship	Mike Moeller
18.	Gorgas Scholarship Program	Ellen Buckner
19.	Electronic Media	Brian Toone
20.	Development	Larry Krannich

D. Old Business .....

E. New Business .....

F. Adjournment

Minutes of the Fall 2011 Executive Meeting

1. Meeting was called to order by President Mickie Powell at 8:05 am and the minutes from the spring Exec. Comm. meeting were approved.
2. Local Arrangements Committee – Prakash Sharma gave a report on the annual meeting that will be at Tuskegee Univ. in the Kellogg Center on February 22-24, 2012. JAS will meet in the Engineering Building (registration on February 23 and meeting on February 24). Exec.

Comm. meeting on February 22nd with the dinner at 6:30 pm at a cost of $15. Dr. Sharma gave a presentation on the facilities, local arrangements for AAS and JAS, food for breakfasts and lunches, and overall schedule. Rooms are equipped with computers and projectors. Steve Ricks, State AMSTI Director, is being planned as the speaker for the Friday AAS/JAS/Gorgas lunch. Gorgas and JAS students will have a poster session prior to the banquet on Thursday evening. All Senior Academy posters will be displayed from 11:30 AM to 1 PM on Thursday with a box lunch served. Room costs at the Kellogg Center are $99 for single and double rooms. On-line registration will occur on the AAS website using PayPal with a deadline that matches the room reservation deadline (February 10th). After the deadline, non-student registration will will increase by $20 and student by $10..

3. Michael Longmire and Zac Ingram made a presentation on their UAB Science and Technology Honors project to foster more participation in undergraduate research presentations at the Academy meeting. This year they will hold a book award. Next year they will have a monetary award sponsored by Alabama industries and/or organizations that could be scholarships, research support, etc. They were encouraged to contact CUR (Council for Undergraduate Research) and to investigate if their home institution would participate in an award for the student. The Exec. Committee moved to encourage their efforts.
4. Mickie Powell discussed the ongoing efforts to strengthen the Junior Academy and engage more high school students in the Junior Academy competitions. Efforts are underway to interface with AMSTI infrastructure to accomplish this. An Ad Hoc committee was proposed to work with the AJAS and the Junior Science and Humanities Symposium to address the long term issues identified:
   - Identify strengths and weaknesses of the system
   - Identify people and resources located in each region
   - Take an active role in the restructuring of the current districts
   - Increase visibility
   - Be involved in teacher recruiting and training in each area.
5. Catherine Shields discussed the Junior Academy and her efforts to increase the number of students in the JAS competitions. She encouraged Academy members to attend local and regional science fairs to make contact with students and encourage them to participate in JAS.
6. Ellen Buckner presented the need for funding to support the Gorgas Teacher Fellow. The Exec. Committee agreed that this funding should come from the JAS budget. AMSTI has accepted the Gorgas Scholarship program as an AMSTI Affiliate.
7. Safaa Al-Hamdani informed the committee that the January 2011 issue of the Journal was printed at JSU at a cost of $600 for printing and shipping. He asked for a decision regarding whether to continue printing or to convert to only electronic editions. The committee approved to continue printing and posting of the electronic edition. After reviewing the Journal site, the committee recommended that reviewer names not be posted with the respective Journal issue, but display as a year aggregate on the Journal homepage.
8. Dues renewal requests need to be sent by the Secretary.
9. Travel awards need to be restricted to travel and not be used for registration fees. The committee recommended that $15 vouchers be issued to students so they pay a reduced registration fee. Students from the host institution would only be eligible for the vouchers and would not receive other travel funds. All students receiving travel awards must pre-

register. The Research Committee is charged with the responsibility to develop guidelines for the awards and publish these on the website.

10. The Long-Range Planning committee recommends that the Development Committee be activated to be involved in fund raising. The President will make two Academy member appointments to this committee.
11. A chair and an additional member is needed for the Date and Place of Meeting Committee. Contacts will be made to find a chair. A host for the 2013 meeting needs to be located. Suggestions focused on institution in the Birmingham area.
12. A suggestion was made to put the list of Gardner award Fellows recipients on the Academy website. In addition the nomination forms will be posted.
13. All the reports from the officers, sections, and committees were reviewed in the order presented in the agenda.

The meeting was adjourned at 11:45 am.

A

1. Call to Order

2. Review/approval of minutes of the Spring 2011 Executive Committee Meeting

MINUTES OF THE
ALABAMA ACADEMY OF SCIENCE
SPRING EXECUTIVE COMMITTEE MEETING
JACKSONVILLE STATE UNIVERSITY
WEDNESDAY, March 2, 2011, 6:00 PM, Rm 111, MARTIN HALL

See April 2011 Issue of the Journal of the Alabama Academy of Science

B. Officer Reports

B-1

Board of Trustees Report

No Report Submitted.

B-2

President's Report

I have been working with Ellen Buckner, Catherine Shields, Ronald Hunsinger, Adriane Ludwick, and Larry Krannich to increase the involvement of the senior academy members with the activities of the junior academy. I have sent a letter to all active members inviting them to participate in local science fairs and inform students about the opportunities available through the junior academy and the Gorgas competition for scholarship monies. Please feel free to forward the letter to any interested parties.

It was decided that working with the Alabama Math, Science, and Technology Initiative would be the most effective way to reach students and teachers with information about the local competitions and resources. Ronald Hunsinger and I will be meeting with Steve Ricks, the State Director for Alabama Math, Science, and Technology Initiative to inform him about the work of the AAS and to show our support for the junior academy, the state science fairs and paper competitions. We will also be inviting him to speak to the junior academy members and Gorgas competitors at the Friday lunch at this years meeting, if this can be accommodated by the local arrangements committee.

It is my hope that we can transition these students into active members of the Alabama Academy of Science as they move into research at the university level.

Respectfully Submitted,

Mickie Powell
President AAS

Action Item
Establish an Ad Hoc committee to work with the AJAS and the Junior Science and Humanities Symposium (JSHS) to address the long term issues identified by the ad hoc task force above:

- Identify strengths and weaknesses of the system
- Identify people and resources located in each region
- Take an active role in the restructuring of the current districts
- Increase visibility
- Be involved in teacher recruiting and training in each area

B-3

Report of the President-Elect

I have contacted all elected committee chairs and officers in order to survey their work, identify any existing concerns/problems and to offer assistance. In most cases, the chairs reported that the committees are operating as expected, i.e., business as usual. More detailed reports of their committee work can be seen in their individual reports. In particular, I conferred with Dr. Emanuel Waddell, Second Vice-President, about his upcoming duties as Chair of the Nominating Committee and offered him my full support in this task. I wish to also express my appreciation for the leadership of President Mickie Powell.

Over the summer, an *ad hoc* committee was formed to discuss some issues related to the AJAS. Dr. Catherine Shields' willingness to remain the State Coordinator through this year is greatly appreciated along with her organization and leadership skills. Her work in arranging for continued AJAS national student competitions is especially appreciated. The *ad hoc* committee also met several more times *via* phone conference to discuss the AJAS further and to consider a replacement nominee for Dr. Shields (at her request). This committee also discussed ways to strengthen and increase membership/participation in the AJAS.

The other major committee item needing our attention is the replacement of Dr. George Cline as Chair of the Research Committee. Dr. Cline has requested this due to an unexpected illness his wife is experiencing and his increased responsibilities as Acting Chair of his department while Dr. Romano deals with a health issue. I speak for the whole academy in wishing the best for George in these situations. His contributions to the Academy have been vital, and we look forward to his return to active leadership in our organization. As a result of this request, the nominating committee brings as an **ACTION ITEM** the nomination of Dr. Brian Burnes as a member and Chair of the Research Committee.

Respectfully Submitted,
Ronald N. Hunsinger

Action Item

- Nomination of Dr. Brian Burnes as a member and Chair of the Research Committee.

B-4

Second Vice President Report

As Second Vice President, I have received information from Ronald Hunsinger, President Elect of the Alabama Academy of Science concerning the duties of the Second Vice President. I have acclimatized myself with the current listing of officers and familiarized myself with the constitution and bylaws.

Respectfully submitted,

Emanuel Waddell

B-5

Secretary Report

See the minutes of the spring Executive Committee and AAS general meeting in the April 2010 issue of the *Journal of the Alabama Academy of Science* and as posted on the Academy website.

Respectfully submitted,
Janie Gregg

B-6

Treasurer's Report

The Treasurer's Report for all the Academy accounts is as follows:

Respectfully submitted,
Bettina Riley

TREASURER'S REPORT
ALL ACADEMY ACCOUNTS
2/23/2010 through 9/29/2011 (Cash Basis)

CHECKING ACCOUNT			**Current Balance:**	**$ 1,759.58**
Category	*2/23/2010- 9/29/2010*	*2/23/2011- 9/29/2011*	*Amount Difference*	
INCOME				
Chem Symposium Support	$ 384.92	$ -	$ (384.92)	
Gale Group Royalty	$ 121.42	$ 77.70	$ (43.72)	
JAAS Subscriptions	$ -	$ 150.00	$ 150.00	
JAAS Support	$ 250.00	$ 100.00	$ (150.00)	
JAS Support	$ -	$ 1,255.00	$ 1,255.00	
Membership Dues	$ 5,935.71	$ 3,429.11	$ (2,506.60)	
Other Income (Business)	$ -	$ 2,000.00	$ 2,000.00	
Publication Income	$ 400.00	$ 500.00	$ 100.00	
Science Fair	$ 31,540.00	$ 24,310.00	$ (7,230.00)	
Transfer From CD	$ 5,000.00	$ -	$ (5,000.00)	
TOTAL INCOME	$ 43,642.05	$ 31,821.81	$ (11,820.24)	
EXPENSES				
Audit Fee	$ -	$ 475.00	$ (475.00)	
Bank Charge	$ -	$ 123.98	$ (123.98)	
Gardner Award Plaque	$ -	$ 226.74	$ (226.74)	
Honorarium	$ 3,500.00	$ 3,500.00	$ -	
Honorarium and Expenses	$ 27.45	$ -	$ 27.45	
JAAS Honorarium	$ 2,025.00	$ 1,000.00	$ 1,025.00	
JAAS Mailing	$ 142.48	$ -	$ 142.48	
JAAS Printing	$ -	$ 3,741.79	$ (3,741.79)	
Mason Scholarship	$ 1,000.00	$ 1,000.00	$ -	
NAAS Dues	$ -	$ 99.50	$ (99.50)	
Office Expenses (Business)	$ 735.30	$ -	$ 35.30	
PaperPoster Award	$ 1,890.00	$ 100.00	$ 1,790.00	
Printing and Reproduction (Business)	$ -	$ 20.50	$ (20.50)	
Research Grant	$ -	$ 800.00	$ (800.00)	
Science Fair INTEL	$ 26,908.71	$ 26,030.00	$ 878.71	
Travel Award	$ 1,750.00	$ 120.00	$ 1,630.00	
Web Fees	$ -	$ 179.20	$ (179.20)	
Wire Transfer	$ 48.00	$ -	$ 48.00	
TOTAL EXPENSES	$ 38,026.94	$ 37,416.71	$ 610.23	
OVERALL TOTAL	$ 5,615.11	$ (5,594.90)	$ (11,210.01)	

SAVINGS ACCOUNT			**Current Balance:**	**$ 2,500.05**
ACTIVITY				
Interest Inc	0	2.15	2.15	
Other Income (Business)	0	2,500.00	2,500.00	
Transfer From Savings to Checking	0	-1,265.23	-1,265.23	
TOTAL INCOME	0	1,236.92	1,236.92	
OVERALL TOTAL	0	1,236.92	1,236.92	

CD 1		**Current Balance:**	**$ 7,453.10**
CD 2		**Current Balance:**	**$ 10,545.27**
JAS CHECKING ACCOUNT		**Current Balance:**	**$ 7,740.02**
GORGAS CHECKING ACCOUNT		**Current Balance:**	**$ 397.00**
	TOTAL ALL ACCOUNTS		**$ 30,395.02**

B-7

Journal Editor Report

The following has been accomplished since the last meeting:

- The Alabama Academy of Science Journal Vol. 82. No 1 has been successfully released. In both formats, electronically and hard copy.
- The Alabama Academy of Science Journal Vol. 82. No 2 has been electronically released. However, the hard copy has been delayed.
- I would like to bring to the attention to the members of the academy to become more active in submitting papers to the journal and volunteering to review manuscripts.
- I have met with Stephen Wallace, the Executive Sales Representative for Cengage Learning - Brooks/Cole Pub. and he informed me that they are willing to continue to advertise in the journal.
- I would like to emphasize the importance of establishing successful communication between the editor and both the secretary and the treasury too best serve the Academy.

Respectfully submitted,
Safaa Al-Hamdani

B-8

Interim Counselor to Alabama Junior Academy of Science Report

A total of eighteen students representing four regions presented papers at the state competition at Jacksonville State University March 3-4, 2011. Ten students from the Central region, seven students from the South Central region, one from the Southeast region, and one student from the Northwest region presented papers at the state competition. State winners were:

First place: Pranjal Gupta (Auburn), sponsor Mark Jones
Second place: Shawn Tuteja (Altamont), sponsors Mary Williams and Donna Kentros
Third place: Peter Johnsen (Altamont), sponsors Mary Williams and Donna Kentros
Fourth place: Olivia Dure (Altamont), sponsors Mary Williams and Donna Kentros
Fifty place: Mary Wills (JCIB), sponsors Catherine Shields and Marilyn Niemann

After the resignation of Henry Barwood in the spring of 2011, Catherine Shields accepted the position of Interim State Counselor. It was subsequently discovered that Dr. Barwood had failed to submit the grant application to the Junior Sciences and Humanities Symposium (JSHS), therefore the Alabama delegation to the national meeting was not funded. Members of the Alabama Academy of Science (AAS) graciously

gave from their personal finances to fund student participation in the national competition. AAS also agreed to support the work of AJAS from their budget to assure that promises were kept to student

participants. I cannot adequately express both my personal gratitude and the gratitude of AJAS participants for this sacrificial show of support for the people and the mission of AJAS. The gracious, willing sacrifice demonstrated by the individuals comprising AAS was both moving and inspiring. Subsequent to these events, the national office graciously funded expenses for the top three students to attend the national meeting, along with one chaperone. Peter Johnsen and Olivia Dure were unable to attend the national meeting, enabling the remaining state winners to attend the national meeting fully funded. AJAS remains extremely grateful to both the members of AAS and to JSHS for supporting the program and the people of AJAS.

The national Junior Sciences and Humanities Symposium (JSHS) took place April 27 – May 1, 2011 in San Diego, California. Pranjal Gupta, Shawn Tuteja and Mary Wills attended with Catherine Shields chaperoning. Shawn has been a participant in AJAS for four years, and is currently attending Stanford University. Mary is a freshman honors student at the University of Alabama. Pranjal is a senior at Auburn High School and was elected President of AJAS.

Despite efforts to spread the word about AJAS, participation in the program remains low. Although the Northwest region has been active for many years, Vicki Farina was unable to attend this year and has been unable to recruit another teacher to sponsor student papers. One of her students attended the meeting with her mother. The Central region remains active under the leadership of Mary Williams and Catherine Shields. Virginia Vilardi in the South Central region is also active. Mary Thomaskutty is the regional coordinator for the North region, but has not attended the state meeting for many years and has not submitted any student papers.

Work to expand participation in the AJAS Paper Reading and Gorgas competitions continues through the work of Dr. Mark Jones, AJAS Fellow. Dr. Jones will host a booth at the Alabama Science Teachers' Association meeting October 18, 2011 at the McWane Science Center in Birmingham. Previous attempts to expand the program include distribution of fliers at teacher meetings and student science competitions; e-mails, letters, and phone calls to teachers; and maintenance of a facebook page and the AJAS website. Mickie Powell, AAS President, sent a letter in September 2011 to AAS members requesting they attend their local science fairs for the purpose of visiting posters to meet student participants and encourage them to enter both AJAS Paper Reading and Gorgas competitions. All AAS members are encouraged to seek networking opportunities with our target population of high school students interested in scientific research. Students competing in science fair have already completed the project that can be submitted to additional competitions, such as Paper Reading and Gorgas, in hopes of earning scholarship funds. AAS members can help students realize this opportunity and fund their education.

Members of the AAS and AJAS have met by conference call to plan future initiatives for the program. Dr. Ellen Buckner, Gorgas Chair, met recently with Steve Ricks, Alabama Math, Science, and Technology Initiative (AMSTI) Director to discuss a partnership between AJAS and AMSTI. AMSTI funds positions for experienced teachers to serve as resources for classroom teachers. As AMSTI personnel are in touch with teachers throughout the state, it would be an efficient method for promoting the mission of AJAS through AMSTI personnel. The idea was well received by Steve Ricks. Mickie Powell, Catherine Shields, and others will pursue meeting further with Steve Ricks in October, 2011.

In September, Catherine Shields submitted a grant to JSHS for AJAS to sponsor the JSHS program in the state of Alabama. As the funding amount has remained at $9,600 for many years, Dr. Shields requested an

increase to $12,000 for 2011-12. The increase was granted, with a question about the per capita cost of the program and encouragement to increase participation in AJAS.

The bank account of AJAS was recently moved from Troy, AL to Compass Bank in Birmingham. Catherine has requested that BJ Bateman, State Counselor prior to Henry Barwood, close the account in Troy and send any remaining funds to her for deposit in the Birmingham account.

I remain committed to the AJAS program. However, because the IB teaching schedule and the needs of AJAS peak simultaneously in the spring, I decline the gracious offer of the AAS to be the permanent State Counselor. I continue to function in this role and, along with other AAS members, hope to appoint a permanent State Counselor in the near future. I will continue to serve as a resource person for the new State Counselor and will serve as Associate State Counselor.

Respectfully submitted,
Catherine Shields,
Interim State Counselor, AJAS

B-9

Science Fair Coordinator Report

Seventeen high school students from across the state of Alabama traveled to Los Angeles, California to the 62nd International Science and Engineering Fair. Fourteen came to compete, three came to observe and learn. Intel/ISEF is the world's largest pre -college science and engineering competition. Over 1600 students from 59 countries competed in this event that culminates a year of research and experimentation by these young scientists. The awards are given by category (16 different) or as special awards from over 60 participating organizations.
The 2011 winners from the State of Alabama were:

Special Awards Received:

Acoustical Society of America
Certificate of Honorable Mention, Each winner will also receive a one-year ASA membership
Precision Location of Acoustic Sources, Alexander Nathan Finney, 16, Covenant Christian Academy, Huntsville, Alabama

AVASC-Ashtavadhani Vidwan Ambati Subbaraya Chetty Foundation
Second Award of $500 U.S. savings bond
Developing More Efficient Models of Titanium Dioxide Dye-Sensitized Solar Cells, Shawn S. Tuteja, 18, The Altamont School, Birmingham, Alabama

American Psychological Association
Third Award of $500
Brain Plasticity: The Effect of Age (A Two Year Study, Olivia A. Dure, 16, The Altamont School, Birmingham, Alabama

IIT College of Psychology
Renewable Scholarship to the IIT Institute of Psychology $15,000 per year for up to four years
W, Wh, Why Can't I Have the Job? Listeners' Perceptions of People Who Suffer from Speech Impediments, Rosalyn Jules Langhinrichsen-Rohling, 14, S. S. Murphy High School, Mobile, Alabama

U.S. Patent and Trademark Office Society
The Effect of Heavy Metals on Porphyrin Ring Compounds Part Three: Leached Mercury into the Base of the Food Chain near Coal Combustion Waste Disposal Sites, Arina Ghosh, 17, Alabama School of Fine Arts, Birmingham, Alabama

Society of Exploration Geophysicists
For projects that display excellence related to the geophysical sciences.
Certificate of Honorable Mention
Precision Location of Acoustic Sources, Alexander Nathan Finney, 16, Covenant Christian Academy, Huntsville, Alabama

Wolfram Research, Inc.
This award was presented to all of the finalists and observers from Alabama. The finalists were: Courtney Kloske, Joel Tinker, Ellen Price, Arina Ghosh, Alexander Finney, Rosalyn Jules Langhinrichsen-Rohling, Shawn S. Tuteja, Olivia A. Dure, Marie Reuter, Lakshmi Raju, Lindsay Grizzard, Rachel England, Rakesh Goli, and William Davis Haselden. The observers were: Elizabeth Sudeiha, Jodie Tinker, and Megan Moody.

Respectfully submitted
Virginia Vilardi

B-10

Alabama Science Olympiad Report

Registration is in full swing! Nationals this year will be close, University of Central Florida.

As for A2, the little Olympians, we had over 60 teams registered last year. Tournaments included University of West Alabama, Auburn University and Jacksonville High School, and they have their dates already set for 2011-2012. Still need a host for an A2 tournament in the Birmingham area.

If we can keep the present folks hosting tournaments (three A2 tournaments, four B tournaments, and four C tournaments) and add a few more, Alabama Science Olympiad numbers could rebound. In addition to the current regional hosts (UWA, AU, JHS, UAB, UAH, UAT, and SHC), I am very appreciative of directors and event supervisors at Huntingdon College as they have agreed to host both B and C State March 10, 2012.

Please consider showcasing your campus, staff and students, and the science department by hosting a tournament! As always, thank you for your support of Alabama Science Olympiad. Alabama Science Olympiad web page: http://aso.jsu.edu/ The following is the schedule of tournaments for 2011-2012.

ALABAMA SCIENCE OLYMPIAD 2011-2012

Division A2 Grades 3-6 Olympiad Tournaments

University of West Alabama, Tuesday, Oct 18. Dr. Janis Beard, Univ. of West Alabama, Station 7, Livingston, AL 35470.

Jacksonville High School. February 18. David Peters, 1000 George Douthit Drive SW, Jacksonville, AL 36265. (256) 435-4177, www.esoatjhs.org

Auburn University, March 31. Greg Harris & Terry Tidwell, Department of Mathematics, 218 Parker Hall, Auburn, AL 36830 harriga@auburn.edu or tidweto@auburn.edu

Division B Grades 6-9 Olympiad Tournaments

Spring Hill College, March 3. Dr. Carolyn R. Simmons, Assistant Professor of Chemistry Spring Hill College, 4000 Dauphin Street, Mobile, AL 36608 csimmons@shc.edu

Auburn University, February 25. Dr. Steve Stuckwisch, Department of Geology, 108 Tichenor Hall, Auburn University, AL 36830. (251) 844-6575 sstuckwisch@charter.net; http://www.auburn.edu/~stuckse/ScienceOlympiad/

University of Alabama in Huntsville, February 18. Mrs. Vanessa Colebaugh, 5019 Willow Creek Drive, Owens Cross Roads, AL 35763. (256) 922-5747 nessacita@comcast.net, http://www.uah.edu/sciolympiad/index.php

University of Alabama. February 18. Luoheng Han, Ph.D., Associate Dean and Professor, College of Arts and Sciences, University of Alabama, Box 870268, Tuscaloosa, AL 35487-0268, Phone: 205.348.7007, Fax: 205.348.0272. www.as.ua.edu

Division C Grades 9-12 Olympiad Tournaments

Spring Hill College. March 3. Dr. Carolyn R. Simmons, Assistant Professor of Chemistry Spring Hill College, 4000 Dauphin Street, Mobile, AL 36608 csimmons@shc.edu

University of Alabama at Birmingham. February 18. Miss Charlotte Kent, Box 210, Blount Hall, 1001 14th St. S. Birmingham, AL 35205. cmckent@uab.edu

University of Alabama in Huntsville. February 18. Mrs. Vanessa Colebaugh, 5019 Willow Creek Drive, Owens Cross Roads, AL 35763. (256) 922-5747 nessacita@comcast.net, http://www.uah.edu/sciolympiad/index.php

University of Alabama. February 18. Dr. Luoheng Han, Associate Dean and Professor, College of Arts and Sciences, University of Alabama, Box 870268, Tuscaloosa, AL 35487-0268, Phone: 205.348.7007, Fax: 205.348.0272. www.as.ua.edu Becky Snow Bsnow@eng.ua.edu

State Science Olympiad Tournaments

Huntingdon College. State B and C. March 10, 2012. Dr. Sidney Stubbs, Assoc. Vice President for Institutional Assessment and Compliance and Professor of Mathematics sstubbs@huntingdon.edu and Dr. Jim Daniels, Assoc. Prof. of Biology, jdaniels@huntingdon.edu , 1500 E Fairview Ave, Montgomery, AL 36106 (334) 833-4430

2012 Science Olympiad National Tournament. May 18-19, 2011, University of Central Florida.

Respectfully submitted,
Jane Nall

B-11

Counselor to AAAS Report

The Annual Meeting is one of the most widely recognized pan-science events, with hundreds of networking opportunities and broad global media coverage. An exceptional array of speakers will gather at the 2012 AAAS Annual Meeting from 16-20 February in Vancouver, B.C.

The theme of "Flattening the World: Building the Global Knowledge Society" is intended to focus the program on the complex, interconnected challenges of the 21st century and on pathways to global solutions through international, multidisciplinary efforts.

Once annually, AAAS sponsors an international conference — four days of symposia, lectures, seminars, workshops, and poster sessions that cover every area of science, technology, and education. Typically attendees hail from nearly 60 countries, and everyone is welcome. Those who join us will have the opportunity to choose from among a broad range of activities, including plenary and topical lectures by some of the world's leading scientists and engineers, career development workshops, and an international exhibition.

Please check out the website at www.AAAS.org. Contains up to date information on science and education news.

We welcome the opportunity for any AAS member to attend the AAAS meeting on our behalf. Information about the AAAS can be obtained at www.aaasmeeting.org.

Respectfully submitted,
Stephen A. Watts

B. 12. Section Officers

B-12, I

Biological Sciences Section Report

The final numbers for the 2011 AAS meeting at Jacksonville State University were:

A total of 39 presentations were made by faculty and students.
19 talks (1 no show)

19 posters (1 no show)

6 students were entered in the competition for best talk. The award for the best talk went to:

Is there a dietary requirement for ascorbic acid in *Lytechinus variegatus*? *Warren T Jones*, Stephen A Watts, Laura Heflin, Anthony Siccardi, III, Mickie L Powell, Victoria K Gibbs, Hugh S Hammer, and Addison L Lawrence, University of Alabama at Birmingham.

9 students were entered in the competition for best poster. The award for the best poster went to:

Expression analysis of a potential male sex determining factor in a turtle with temperature-dependent sex determination. *Kayla L Bieser* and Thane Wibbels, University of Alabama at Birmingham.

As mentioned in the 2010 annual AAS report, we feel that it is perhaps unfair to have undergraduates competing with graduate students in these competitions. In cases where there are more than 12 entries in competitions, we'd like to give an undergraduate award and a graduate award for papers and/or posters.

Respectfully submitted,
Katia Shumaker

B-12, II

Chemistry Section Report

The chemistry section participated in the Alabama Academy of Science 2011 Annual Meeting. In addition to the regular Chemistry session (II) that took place on Thursday, March 3rd, 2011 the section participated in a Joint session with the Southeast Society for Environmental Toxicology on Friday, March 4th, 2011. The regular session was attended by approximately twenty presenters and attendees. There were a total of five presenters in the regular session. There continues to be a good representation of undergraduate and graduate presenters in the session. Several presentations resulted in extended discussion. First, second, and third places were awarded to the participants.
The business session included the election of Emanuel Waddell and Nixon Mwebi as Chair and Vice-Chair of the chemistry section.
Several undergraduate students participated in the poster session. Judges were composed of members of the Chemistry section of the Alabama Academy of Science and first, second, and third places were awarded.

Respectfully submitted,
Emanuel Waddell

B-12, III

Physics and Mathematics Section Report

I am happy to report that total twenty seven presenters (Ten oral and seventeen poster Presentations) have submitted their research work for presentation in Physics and Mathematics Section, this year.

Three oral presentations and twelve poster presentations have been entered for student award competition.

Respectfully submitted,
Mohan Aggarwal

Engineering and Computer Science Section Report

We are pleased to have received 11 paper submissions and 4 poster submissions from a broad range of topics for our 2011 Section meeting.

Respectfully submitted,
Qichao Liu

Social Sciences Section Report

No Report Submitted.

Anthropology Section Report

No Report Submitted.

B-12, VII

Science Education Section Report

No Report Submitted.

B-12, VIII

Industry, Environmental, and Earth Science Section Report

No Report Submitted.

B-12, IX

Health Sciences Section Report

No Report Submitted.

B-12, X

Bioethics & History,/Philosophy of Science Section Report

No Report Submitted.

B-13

Executive Director Report

Since March, 2011, I have been involved in the following activities as the Executive Director of the Alabama Academy of Science:

1. Distributed the Local Arrangements Manual to the local arrangements committee at the Tuskegee University to assist them concerning arrangements, program booklet needs, and deadlines associated with the annual meeting of the Academy to be held on the Tuskegee University campus, February 22-24, 2011 with the Executive Committee meeting on February 22nd.
2. Participated in the *ad hoc* committee conference calls to discuss issues related to AJAS.
3. Prepared letters for distribution in late October to Alabama colleges and universities to solicit financial support for the Journal.
4. Prepared the Call for Papers for the 89th meeting of the Academy that will be distributed to all Section Chairs in hard and electronic copy after November 15th.
5. Met with the auditors who audited the Academy accounts.
6. Prepared IRS Form 1023 to file for reinstatement of the Academy's 501(c)3 status that was revoked.
7. Consulted with Brian Toone, Editor for Electronic Media, to update the website with 2011-2012 officer and committee lists and to re-activate the on-line submission of Executive Committee reports and generate a compiled document for distribution to all attendees at the meeting.
8. Developed a doodle.com site for intended participation in the Executive Committee breakfast and meeting.
9. Prepared the committee chair report compilation and action items for distribution at the Fall Executive Committee meeting.

Respectively submitted,
Larry K. Krannich

C. Committee Reports

C-1

Local Arrangements Committee Report

This report will be an oral report at the Executive Committee Meeting on Saturday, October 8, 2011.
P. C. Sharma, Chair, Local Arrangements Committee

Finance Committee Report

The assets of the Academy as reported at the Fall Executive Committee meetings and Annual Spring meetings since 2001 are listed below.

Period	Assets (End of Period)	Change	Period	Assets (End of Period)	Change
1/1 – 10/12/2001	$71,763		1/1 – 12/31/2001	$75,813	
1/1 – 10/12/2002	$72,197	$434	1/1 – 12/31/2002	$72,813	–$3,000
1/1 – 10/12/2003	$71,403	–$794	1/1 – 12/31/2003	$74,800	$1,987
1/1 – 10/26/2004	$74,265	$2,862	1/1 – 12/31/2004	$74,610	–$190
1/1 – 10/26/2005	$63,895	–$10,370	1/1 – 12/31/2005	$65,561	–$9,049
1/1 – 10/26/2006	$62,162	–$1,733	1/1 – 12/31/2006	$67,555	$1,99
1/1 – 10/31/2007	$34,004	–$28,158	1/1 – 12/31/2007	$36,435	–$31,120
1/1 – 10/10/2008	$25,618	–$8,386	1/1 –-3/13/2009	$28,989	–$7,446
1/1 – 10/14/2009	$26,937	$1,319	1/1 – 3/23/2010	$26,814	--$ 2,175
1/1 – 10/1/2010	$22,144	--$4,793	1/1 – 2/14//2011	$24,865	--$1,949
1/1 – 10/1/2011	$21,668*	--$476			

Our finances may be stabilizing but are at a decade low. In view of this and the possible need to additionally support the JAS, the Academy needs to maintain realistic budgets to reflect this and should be ready to consider steps (i.e., dues increase, increased meeting registration fees, etc.) to augment revenue in the near future.

Ken Marion
Chair, Finance Committee

*Does not include $397 in Gorgas checking account and $7,740 in JAS checking account. These accounts have not been included in the table above during previous years; thus, for comparative purposes, they were not included this year.

Membership Committee Report

No Report Submitted.

C-4

Committee on Research Report

No Report Submitted.

C-5

Long-Range Planning Committee

The following recommendations to strengthen the Academy were generated by the Long-Range Planning Committee at the 2011 Annual Meeting of the Academy.

- **The Academy should initiate fund raising for the Mason Scholarship.** The Executive Committee should establish a committee to develop a mechanism for a successful and ongoing fund raising project. The goal should be an endowment fund sufficiently large to generate at least one Mason Scholarship on an annual basis. The use of PayPal, or something analogous to PayPal, is recommended (If PayPal is used, then it will need to be streamlined for donations.). A campaign reaching non-academy individuals, philanthropic organizations, industries, etc. will need to be organized. The fund raising committee will need to develop a realistic timetable for this fund raising activity.

- **A committee should be established that focuses on the role of the Academy as related to the Alabama Junior Academy of Science (AJAS), the Science Olympiad in Alabama, the Science Fairs in Alabama and the Gorgas competition.** Concerns of this committee should include the financial status of each as related to the Academy, how to involve an increased number of teachers in these activities so as to strengthen each activity, how to publicize each activity and its relationship to the Academy, and what role the Academy can play so as to strengthen both financial needs and personnel needs of each activity.

Respectively submitted,
Adriane Ludwick, Chair
Anne Cusic
Ken Marion
Eugene Omasta

C-6

Auditing, Senior Academy Committee Report

A full audit of the Senior Academy accounts was conducted in Spring 2011 and the Treasurer's Report reflects the status of all accounts. The audit determined that not all Academy accounts were being reported on the IRS 990 form and that form had not been filed for the past five years, which resulted in the revocation by the IRS of the Academy's 501(c)3 status.

I have contacted the Secretary, Bettina Riley. In January, 2012 Ms. Riley will make the Academy's 2011 financial records available to me. I will carefully review the records and will submit an auditor's report before the spring meeting.

Respectfully submitted,
Robert Angus

C-7

Auditing, Junior Academy Committee Report

No Report Submitted.

C-8

Editorial Board & Associate Journal Editors Committee Report

No Report Submitted.

C-9

Place and Date of Meeting Committee Report

Currently there are no volunteers to host future annual meetings of the Academy beyond 2012. We need a list of future hosts at least through 2013 to assure adequate planning for future meetings.

C-10

Committee on Public Relations Report

No Report Submitted.

C-11

Archives Committee Report

We need to obtain photographs (especially of members of the Executive Committee), committee reports, minutes of the AAS Executive Committee meetings, and any other materials that may be of interest to our membership. Items that may not seem of interest at present may be of great interest in the future. Photographs of officers and members at meetings are of special interest.

If you have items that you believe may be worthy of inclusion in the AAS Archives, please send them to me or to Dr. Dwayne D. Cox, University Archivist, Auburn University Ralph B. Draughon Library, 231 Mell Street, Auburn University, AL 36849.

Access to our AAS Archives is available 7:45-4:45 Monday-Friday. Dr. Cox has provided the following information relative to access. Archives materials **do not** go out on interlibrary loan. Patrons can come in and use them according to the donor specifications. Some require special permission from the donating office or persons who made the donation or sometimes the archivist. Materials to be used at night or weekends need to have special arrangements made so they can be pulled before 4:30 in the afternoon (Friday afternoon for weekend use). Copies can be made in most cases and that can be done either by going through InfoQuest or contacting Dr. Cox or the reference desk at 334/844-1732.

I encourage all officers and members of the AAS to donate significant documents, photographs, etc. to the archives.

Sincerely,

Troy L. Best
Archivist

C-12

Committee on Science and Public Policy Report

In Alabama, a number of issues of science and education sporadically appear during the legislative sessions. These include various attempts to introduce creationism into the pre-college public school system, to diminish the pervasive presence of evolution in science textbooks, or to cast doubt on evolutionary science.

I'll summarize below a number of developments on these issues this past year.

Creationism in the public schools

A news story during the spring about bibles distributed at the Blues Springs Elementary School (Limestone County) also reported a claim of the teaching of creationism in a fifth grade class during instruction in evolution[1]. In response to this story, a spokesperson with the state Department of Education is reported to have written in an email that "The Alabama Course of Study deals with Theories of

[1] http://www.foxnews.com/us/2011/03/24/alabama-superintendent-denies-claims-bibles-distributed-class/

Evolution… Creationism is one of those theories. The Alabama Course of Study presents each of these so that students can draw their own conclusion for themselves." Of course this is not true – the Alabama Course of Study:Science does not provide for the teaching of creationism, and any instruction in creationism is unlawful (documented by in a letter to the superintendent of Limestone County schools by the ACLU of Alabama[2]. This case is just one of the latest examples of the clashing of local religious standards with public school science instruction.

No legislation concerning science education in Alabama

During the 2009 legislative session, some representatives and senators in the Alabama legislature introduced "Academic Freedom" bills. The language of these bills (copied from similar efforts in other states) identifies them as creationist bills in disguise (but hardly disguised, as descriptor keywords include "creationism, "intelligent design", and "origins"). For unknown reasons, the promoters of these bills did not introduce them in either the 2010 or 2011 legislative sessions, saving us a considerable amount of time and effort compared to the 2009 legislative session.

The cycle of science standards and textbook adoptions in Alabama is interrupted

The "normal" cycle of course standards and textbook adoptions for the K-12 public school systems in Alabama is six years. The state Board of Education directs the processes established by law for the appointment and work of committee members. Normally, during one academic year the curriculum standards governing a course are drafted by members (appointed) of the Course of Study committee, subjected to public review, and ultimately approved by the state Board of Education. During the following academic year, another committee (the State Textbook Committee) is appointed, reviews texts submitted by publishers, and after opportunities for public review, recommends texts to the state Board of Education for adoption. The current science standards was last adopted in the spring of 2005. Textbooks were last adopted 2006, and the list is approved through May 31, 2012.

The National Research Council appointed a committee to draft new framework for science standards, and their report was released July 19, 2011. With the impending release of the report, I understand that the Alabama Dept. of Education held in abeyance the process of revising curriculum standards and science textbook adoptions. The schedule as presently published is for Alabama science standards to be reviewed in 2012-13, with textbooks to be reviewed in 2013-14. I also was told that the new framework for science standards was problematic in part because of its tight integration of evolution in studies of the various sciences. Of course this comes as no surprise to us, given the long history of anti-evolution efforts in Alabama.

So it looks like the "normal" hexennial action on science education in Alabama will not ramp up until next fall.

Respectfully submitted,
Scott Brande

C-13

Gardner Award & Fellows Committee Report

[2] http://www.aclualabama.org/News/PressReleases/Highlights/3.22.11LimestoneCountyLetter.pdf

The ***Wright Gardner Award*** was established in 1984 after the name of the Academy's first president who was a great future looking scientist and educator, to honor individuals whose work during residence in Alabama had been outstanding. Persons nominated for this award have included researchers, teachers, industrialists, clinicians, scholars and active members and office bearers of the Alabama Academy of Science.

This is to request each and every member of the academy to publicize to individuals, heads of departments, deans and provosts of colleges and universities about this prestigious award. Please solicit nominations from individuals and different academic and industrial organizations for this award. You are welcome to nominate either by e-mail or mailing a hard copy.

The nominations should consist of the following documents.

(i) Formal Nomination Letter,
(ii) Vitae and at least three letters of references from peers, administrators and one by an expert in the area of his/her research, and
(iii) One page citation that will be used for presentation of the award.

Anything missing from items (i, ii, iii) may result in rejection of the nomination. The closing date for nominations is January 15th of each year. The award will be presented in the Joint Annual Meeting of Junior and Senior Alabama Academy of Science, on Thursday, February 23, 2012, during the awards banquet.

The ***Fellow of the Alabama Academy of Science*** designation is made by the Alabama Academy of Science to recognize individuals for their contributions in science and for their services to the Academy. AAS members are invited to submit nominations for this award to the chair of the committee not later than January 15th of each year. Members of the committee should encourage AAS members to submit nominations of outstanding persons. Each nomination should consist of a curriculum vitae and documentation substantiating the person's special contribution to science

in Alabama and service to the Academy.
The nominations should consist of the following documents.

(i) Formal Nomination Letter
(ii) Vitae and at least four letters of references from experts in area of his/her research and
(iii) One page citation that will be used for presentation of the award.

Anything missing from items (i, ii, iii) may result in rejection of the nomination. The closing date for nominations is January 15th of each year. The award will be presented in the Joint Annual Meeting of Junior and Senior Alabama Academy of Science, on Thursday, February 23, 2012, during the awards banquet".

Nomination for the Wright Gardner Award and for Fellows of the Academy can be submitted either by e-mail or mailing a hard copy. The address is given below:

Dr. P. C. Sharma, Chair, Wright Gardner & Fellow Award Committee,
Head of Physics Department,
Tuskegee University

Tuskegee, AL 36088.
Phone: (334) 727-8998; Fax: (334) 724-3917
e-mail: pcsharma@tuskegee.edu

Respectfully submitted,
P. C. Sharma

C-14

Carmichael Award Committee Report

The Carmichael Awards Committee has reviewed the articles published in Vol. 81 of Journal of The Alabama Academy of Science.

The following article was selected for the 2010-2011 Carmichael Award:

Brian S. Burnes, Determining Sources of *E. Coli* Pollution in Dry Creek Alabama, *Journal of The Alabama Academy of Science, 81(1),* 12-22.

The committee will review journal articles published in 2011 contained in volume 82 for the 2012 Carmichael Award.

Respectfully submitted,
Richard Hudiburg

C-15

Resolutions Committee Report

No Report Submitted.

C-16

Nominating Committee Report

No Report Submitted.

William H. Mason Scholarship Committee Report

Last spring the Committee reviewed six completed applications for the William H. Mason Scholarship. After assessing all application materials the Scholarship Committee offered the $1000 scholarship to Mr. Cory Goble. Mr. Goble accepted the award and is enrolled in the non-traditional/5th year teaching master's program at the University of Montevallo.

The previous recipients of the William H. Mason Scholarship are:

1990 - 1991	Amy Livengood Sumner	2009 – 2010	(Not awarded)
1991 - 1992	Leella Shook Holt	2010 – 2011	Danielle Morlan
1992 - 1993	Joni Justice Shankles	2011 – 2012	Cory Goble
1993 - 1994	Jeffrey Baumbach		
1994 - 1995	(Not awarded)		
1995 - 1996	Laura W. Cochran		
1996 - 1997	Tina Anne Beams		
1997 - 1998	Carole Collins Clegg		
1998 - 1999	Cynthia Ann Phillips		
1999 - 2000	Ruth Borden		
2000 - 2001	Karen Celestine, Amy Murphy		
2001 - 2002	Jeannine Ott		
2002 - 2003	(Not awarded)		
2003 - 2004	Kanessa Miller		
2004 - 2005	Mary Busbee, Bethany Knox		
2006 - 2007	Kelly Harbin		
2007 - 2008	Michael Hallman		
2008 – 2009	Sheri Sanders Grosso		

The announcement for applications will again be sent soon to deans in colleges of science and colleges of education within Alabama. This announcement and the application form can be found on the Academy website http://www.alabamaacademyofscience.org/mason.php Members of the AAS Executive Committee are encouraged to copy and disseminate this information. Deadline for receipt of applications is February 1, 2012.

Respectfully submitted,
Michael B. Moeller, Chair

Gorgas Scholarship Committee Report

The 2010 Competition was excellent with finalists from six (6) schools. The final press release is attached. In 2010-2011, the Gorgas Scholarship Competition continued to seek ways to grow the organization of science clubs and entrants to the competitions. Mark T. Jones, PhD, NBCT, began the year are the AJAS-Gorgas Teacher Fellow. He presented sessions at the Alabama Science Teacher Association meeting and has worked to develop connections in several areas. His report and upcoming plans are attached.

We are pleased to report that we have been approved an AMSTI Affiliate. This carries with it the opportunity to provide third year experiences for AMSTI teachers. We will be listed in their brochure and promoted through their network. Dr. Jones is planning to host teacher workshop(s) this summer as part of the affiliation. These will focus on encouraging teachers to participate in mentoring students to enter our competitions.

Due to the loss of AJAS funding for the Spring 2011, there was a major reduction in funds for the Gorgas-AJAS Teacher Fellow position. This comes in the middle of the expected 2-year term for Dr. Jones. In consultation with Drs. Powell and Hunsinger, I would like to propose a one-time allocation of $2500 to match the Gorgas contribution to allow us to finish the 2-year term. If the AJAS funding remains consistent, we can look at possible extending the fellowship for future years. [Action item].

The Committee would like to recognize the many outstanding teacher-sponsors of the students. Their work in encouraging students to enter the competition is instrumental to both the success of the program and to the success of the students.

Respectfully submitted,
Ellen Buckner, Chair

Action Item:

- A one-time $2,500 allocation to match the Gorgas contribution for the Gorgas-AJAS Teacher Fellow position.

STATE OF ALABAMA
DEPARTMENT OF EDUCATION

Larry E. Craven
Interim State Superintendent
of Education

September 26, 2011

Dr. Mark T. Jones
1253 Sanders Street
Auburn, Alabama 36830

Dear Dr. Jones:

Congratulations! Upon review of your application, you have been tentatively accepted to become an AMSTI Affiliate, pending the return of the attached paperwork. We are excited about the opportunity to involve your organization in providing continuing professional development to our AMSTI schools. As you know, AMSTI is looking for organizations that offer this high-quality professional development to our year three and beyond schools, and we are pleased that our reviewers believed your organization met all of the criteria as outlined in the application.

The goal of our AMSTI Affiliate program is to provide the best possible professional development that supports the AMSTI philosophy. As your application meets all of the necessary criteria, we would like to take this opportunity to welcome you to our AMSTI Affiliate program. Your professional development offerings will be listed in a brochure that will be shared with our year three and beyond schools.

Please take the time to complete the attachment with the information that you would like to be included in the Affiliate information and return it to Mr. Benjamin Hicks at P.O. Box 302101, Montgomery, AL 36130-2101 or via e-mail at bhicks@alsde.edu. We will be contacting you in the future to review the preliminary draft of the Affiliate information.

Sincerely,

Steve Ricks
AMSTI Director

RSR/BH/DN

Attachment

Report of Mark Jones, AJAS-Gorgas Teacher Fellow

The following activities have been completed or are in progress:

1. Following our application in the spring, we have received a letter notifying us of our approval as an AMSTI affiliate. There is some paperwork to do to finalize the AMSTI affiliate program for teachers this coming summer. This will be designed to be a full day workshop to bring experimental design in to the classroom, extend that based on student interest into outside projects and then science fair projects. Teachers will be oriented on how to take these projects then further to AJAS and Gorgas.

a. A one hour preview of this idea will be presented at the ASTA convention at the end of October.
b. Dates can be set for this summer so that the workshop can be done at Drake Middle School.
c. We may need to discuss arrangements to make this workshop available in other regions. I will need to contact a teacher to host it in their room and we will have to discuss travel arrangements. Target sites would be Mobile, Birmingham, Huntsville and somewhere in west central Alabama.
d. Upon presenting this, I will begin multimedia collection to eventually put pieces of the presentation on the webpage for information purposes and advertising.

2. ASTA convention – I will be giving one session overview of the AMSTI Affiliate workshop and educating about AJAS and Gorgas. I will be present to collect contact information of teachers who might be interested in more information, passing out fliers and advertising our web site.

3. Officers put to work – officers of AJAS will be organized by Pranjal through the new AJAS Facebook page and Gorgas Facebook page. Copies of multimedia information will be posted on these pages and also on YouTube.

a. Officers will populate the page and actively advertised to get friends to join.
b. Officers will complete application form to create AJAS charter memberships at their school.

4. Web site development – I have contacted Brian Toone who has secured a password for me and educated me about how to update the page now.

a. Interviews with Gorgas students will be placed on the page and further Gorgas interviews will be done this year.
b. Interviews with AJAS students will be placed on the page and further interviews will be done.
c. Interviews from the Auburn University Engineering and Science Fair will be done to create multimedia about this level of competition. Examples of projects will be collected to start a multimedia database of ideas.
d. Forms and paperwork have been digitized and will be placed on the webpage as an option.
e. Pranjal Gupta will do a welcome speech for the webpage in general and this will be updated with the new president in spring.
f. A charter membership page will be created with links to Facebook to attempt to get the word out. We will start with officers can go from there.

Respectfully Submitted,
Mark T Jones, PhD, NCT

The Gorgas Scholarship Committee rankings of the finalists of the 2010 Alabama Science Scholar Search held at the meeting of the Alabama Academy of Science at Jacksonville State University, Jacksonville, Alabama were as follows:

The winner of the first-place tuition grant of $4000 was: Ellen Price, 815 Parker Avenue, Gardendale, AL 35071, Jefferson County International Baccalaureate, 6100 Old Leeds Road, Irondale, AL 35210, Teacher-Sponsor, Catherine Shields

First alternate and winner of a tuition grant of $3000 was: Peter Johnsen, 5380 Trace Ridge Circle, Birmingham, AL 35244, The Altamont School, 4801 Altamont Road, Birmingham, AL 35222, Teacher-Sponsor, Donna Kentros

Second alternate and winner of a tuition grant of $2000 was: Shawn S. Tuteja, 5193 Selkirk Road, Birmingham, AL 35242, The Altamont School, 4801 Altamont Road, Birmingham, AL 35222, Teacher-Sponsor, Donna Kentros

Third alternate and winner of a tuition grant of $1500 was: Rodah Wangondu, 3811 Old Shell Road, Mobile, AL 36608, Murphy High School, 100 South Carlen Street, Mobile, AL 36606, Teacher-Sponsor, Lisa Sudeiha

Fourth alternates and winners of a tuition grant of $500 each were: Arina Ghosh, 405 Windchase Way, Birmingham, AL 35242, Alabama School of Fine Arts, 1800 Rev. Abraham Woods Jr. Boulevard, Birmingham, AL 35203, Teacher-Sponsor, Judith Cantwell; Mary Wills, 7362 Gadsden Highway, Trussville, AL 35173, Jefferson County International Baccalaureate, 6100 Old Leeds Road, Irondale, AL 35210, Teacher-Sponsor, Marilyn Niemann

Unranked Finalists:

Katie L. Dulak, 140 Bowden Road, Titus, AL 36080, Wetumpka High School, 1751 Coosa River Parkway, Wetumpka, AL 36092, Teacher-Sponsor, Virginia Vilardi

Mary McClung, 165 County Road 414, Killen, AL 35645, Brooks High School, 4300 Highway 72, Killen, AL 35645, Teacher-Sponsor, Vicki Farina

Ben McCormick, 7815 North Lake Drive, Trussville, AL 35173, Jefferson County International Baccalaureate, 6100 Old Leeds Road, Irondale, AL 35210, Teacher-Sponsor, Catherine Shields

Jessica Stone, 991 Tarilton Road, Titus, AL 36080, Wetumpka High School, 1751 Coosa River Parkway, Wetumpka, AL 36092, Teacher-Sponsor, Virginia Vilardi

The rankings were established by a panel of judges consisting of department heads, deans and professors from many of the leading universities and industries in Alabama. Winners and finalists in the Gorgas Contest receive offers of tuition scholarships to colleges and universities in Alabama for the study of science. The Gorgas Scholarship Program is named for General William Crawford Gorgas, the Alabama physician who conquered yellow fever in the Panama

Canal Zone and later became the Surgeon General of the United States Army. The purposes of the Gorgas competition are to promote interest in science and to aid in the education of promising students.

Information on the annual competition and awards may be found on the website at www.GorgasScholar.org. For further information, contact Ellen Buckner, DSN, RN, Chair, Gorgas Scholarship Competition, ebuckner@usouthal.edu, (205) 910-9877.

C-19

Electronic Media Committee Report

Website Update
I have made a number of routine updates to the website (announcements, etc…) as well as these below:

Fall Executive Report Submission Page
I updated the report submission page to gather reports for this Fall 2011 executive meeting.

Spring Meeting
I updated the website template to display a graphic I created indicating when and where the spring meeting will be.

Online Journal
I have created a beta (test) version of the online journal using the first two journal issues for this year. These issues are currently public at the following URL – http://www.alabamaacademyofscience.org/beta/

Online Membership Application
As of Friday, September 30th, **87 people** have up-to-date memberships through the paypal system. Of these, all but 12 people will have their membership expire by the time of our spring annual meeting.

AJAS Donations
Only one person has used the donate button on the front page of the website. I believe the system is working correctly – does this mean that people aren't finding the button or just unable/unwilling to donate?

Paypal
I updated the powweb accounts so that they may be paid via paypal and went ahead and made the first payment using paypal to pay **$111.24** for one year of website hosting.
The current Paypal balance is **$898.42**.

Respectfully submitted,
Brian Toone

D

D. Old Business

E

E. New Business

This item will be discussed along with the Local Arrangements Report (C1) at 8:30 AM: Students present to discuss the proposal are: Zac Ingram, Michael Longmire, and Alexis Reuschel

1. Students from the UAB Science and Technology Honors Program have proposed a project that would significantly impact the recognition of undergraduate research in Alabama and the paper/poster competitions at the AAS Annual meeting. The overview of the project is to create and present a number of awards to recognize undergraduate research in the state of Alabama and promote participation in the undergraduate competition at the annual AAS meeting. In its first year the goal is to give book awards and a greater monetary prize for excellence in each discipline at the meeting. The focus of these awards is to reflect excellence in different fields of science within the state of Alabama. To do this the awards are to be representative of great Alabama researchers and scientific organizations. In following years it is our hope to develop grant and scholarship agreements with different scientific companies as prizes for the competition.

   Action Item:

   - Approve the proposed project to elevate the stature of and obtained funding for the annual AAS paper/poster competition

F. Adjournment

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